SEPARATION AND ANALYSIS OF HYDROXYAROMATIC SPECIES IN LIQUID FUELS II. COMPARISON OF ArOH IN SRC-II COAL LIQUID, WILMINGTON, CA, PETROLEUM AND OSCR SHALE OIL

J. B. Green, C. A. Treese, S. K-T Yu, J. S. Thomson, C. P. Renaudo, and B. K. Stierwalt

Department of Fuels Research
National Institute for Petroleum and Energy Research
Division of IIT Research Institute
P.O. Box 2128
Bartlesville, OK 74005

INTRODUCTION

Part I of this series, presented in the symposium, "New Applications of Analytical Techniques to Fossil Fuels," describes a general analytical approach for detailed analysis of hydroxyaromatics (ArOH) in fuels (1). An inherent step in development of analytical methods is evaluation of their performance on actual samples. Thus, ArOH concentrates were isolated from a wide spectrum of fuels, chemically derivatized, and analyzed in detail by GC/MS. Results from three of the fuels studied during development of the analytical method are presented here to 1) demonstrate the viability of the analytical methodology and more importantly to 2) contrast the levels and types of ArOH in three distinctly different fuel types: petroleum, shale oil, and liquified coal.

Although numerous compositional studies on one or more fuels of a given type have been published, papers comparing composition of different types of fuels are quite rare. Tomkins and Ho (2) compared levels of selected polycyclic aromatic amines in coal liquids, shale oil and petroleum, and Allen, *et al.* (3), compared overall structural profiles of heavy fractions from a coal liquid, shale oil, petroleum and tar sand. A comparison of the levels of phenol, and C_1- and C_2-phenols in a shale oil and a coal liquid has also been published (4). These three papers are a fairly complete listing of published reports comparing different fuel types.

Prior work on ArOH in individual fuel types has shown coal liquids to contain phenols, indanols/tetralinols, hydroxybiphenyls/hydroxyacenaphthalenes, hydroxyfluorenes, and naphthylphenols (5,6). A study on coal tar cited similar compounds plus dibenzofuranols and pyrenols/fluoranthenols (7). White and Li (8) identified over 20 specific ArOH compounds ranging from 6 to 15 carbons in an SRC-II coal liquid. McClennen, *et al.* (9), studied changes in phenol and indanol distributions induced by hydrotreating, and Scheppele, *et al.* (10), studied ArOH and multihydroxylated aromatics in raw and hydrogenated anthracene oil. Recently, hydroxylated thiophenic compounds and nitrogen heterocycles have also been identified in coal liquids (11,12). Finally, ArOH in coal liquid oils, asphaltenes, and preasphaltenes have been compared (13); the hexane-insoluble fractions contain higher levels of ArOH and generally more polycyclic ArOH.

Shale oil contains mostly phenols, with lesser amounts of naphthols and trace levels of higher-ring ArOH (14-17). Dihydroxyaromatics are also present (16,17).

Petroleum ArOH cover a wide range of structural types and molecular weights from phenol up to highly substituted polycyclic ArOH (18-21). ArOH usually make up less than 10% of the total acidic compound types in petroleum, whereas they are the dominant acidic class in coal liquids and shale oil.

EXPERIMENTAL

Fuels selected for this work were an SRC-II coal liquid from the Fort Lewis pilot plant run 77SR-12 (22,23), an *in situ* produced shale oil, OSCR 76-552, kindly supplied by the Western Research Institute, Laramie, WY, and a Wilmington, CA, petroleum which underwent extensive study during the API-60 project (18,19,24).

The analytical methodology for isolation and analysis of ArOH is described in a separate preprint (1). The results presented here are a composite of the GC/MS analysis of underivatized, silylated and acylated ArOH concentrates; although, as previously explained (1), analysis of acylated ArOH usually gave the most comprehensive data set.

RESULTS AND DISCUSSION

Only qualitative or semiquantitative results are presented, since the relative GC/MS responses of various derivatized ArOH are unknown. The general format for presentation of data is based on the commonly used Z-number convention, where the Z-number of a given ArOH series is defined by its empirical formula: $C_nH_{2n+z}O$. Although the ArOH were analyzed as either acylated or silylated derivatives, all the tables report ArOH composition on an underivatized basis.

Table 1 shows results from the distillation and liquid chromatographic separations to obtain whole acid and ArOH concentrates. The relative abundance of ArOH is coal liquid > shale oil >>petroleum, as expected. Also, the percentage of total acids attributed to ArOH is greatest in the coal liquid and least in the petroleum.

Tables 2(A-I) show the number of isomers separated and detected by GC/MS at each carbon number of the major ArOH Z-series for the three fuels. If a given distillate contained no members of a given series, it is omitted from that section of Table 2.

Results for the Wilmington 370-535° C ArOH are not included in Table 2. Current GC/MS profiles of this fraction do not show sufficient chromatographic resolution to obtain more than a superficial analysis of ArOH present. Acylation did decrease the retention of ArOH present, as expected, but did not improve chromatographic resolution enough for detailed analysis. The few peaks which were resolved corresponded to Z-series ranging from -16 O to -24 O. Fragment ions from these series do not correspond with those present in synfuel ArOH--probably because the Wilmington ArOH are primarily less condensed structures with naphthene rings attached to 1- and 2-ring aromatic nuclei.

Table 3 compares the overall composition of ArOH in the three fuels. All three fuels contain phenols, indanols/tetralinols and members of the -14 O and -16 O series of ArOH. The major differences between the fuels lie in the relative abundances of these series and in the individual structures of ArOH in these and the other series present. These differences are discussed below for each ArOH series.

-6 O

The coal liquid contains the largest overall abundance of phenols as seen by the large proportion (10.3% of the whole oil) of ArOH in the 200-325° C distillate (Table 1) and by the fact that they are the major ArOH type in that distillate (Table 3). The coal liquid GC/MS profile showed large peaks corresponding to individual C_2-C_4 phenol isomers, which in total probably accounted for nearly half of the ArOH present. C_5- and C_6-phenols were relatively minor components in the coal liquid and no C_7- or higher homologs were detected.

The distribution of phenolic homologs in the Wilmington petroleum was similar to that of the coal liquid (Table 2A). However, their overall abundance was lower by about three orders of magnitude, as estimated by the total ArOH present (225-370° C ArOH were 0.03% of the whole crude, Table 1) and the intensity of phenolic GC/MS peaks observed.

On the other hand, the distribution of phenols in the shale oil was markedly different than the other fuels. Species with a total alkyl carbon number up to C_{17} were observed, and mass spectral fragmentation patterns indicated predominantly straight-chain alkyl substituents or substituents with one or two methyl-branches. In total, phenolic species probably accounted for two-thirds of the shale oil ArOH.

-8 O

Indanols/tetralinols were present in all fuels, but were not a dominant series in any fuel. Their abundance was highest in the coal liquid, since they were the second most prevalent type in the 200-325° C ArOH concentrate which comprised 10.3% of the whole SRC-II oil. Based on mass spectral fragmentation patterns (1), indanols dominated tetralinols in all fuels. The predominance of indanols over tetralinols has been reported previously (5,9). Due to the relatively low amounts of naphthols observed in all fuels, especially the coal liquid, it seems likely that this series may be largely derived from hydrogenation of naphthols during coal liquifaction, shale retorting and petroleum maturation processes.

-10 O

This series was not conclusively identified in any fuel. McClennen, *et al.* (9), alluded to the presence of hydroxytetrahydroacenaphthalenes in their study of coal liquid ArOH, and the authors have tentatively identified a -10 O ArOH series of compounds in a marine diesel fuel. Overall, -10 O ArOH compounds appear to be present at low levels or nonexistent in most fuels.

-12 O

Naphthols were positively identified in the petroleum and shale oil ArOH concentrates, but they were not major components in either fuel. Based on the intensity of the GC/MS peaks and overall amount of ArOH present, naphthols comprised a much more significant percentage of the shale oil than any other fuel. Minor amounts of a higher molecular weight -12 O series, corresponding to tricycloalkylphenols, have tentatively been identified in the SRC-II >325° C ArOH concentrate.

-14 O

Compounds in this series were quite prevalent in the coal liquid and petroleum, but relatively minor in the shale oil. As discussed earlier (1), distinquishing between the various isomers of this series on the basis of mass spectral fragmentation patterns is quite difficult. Possible structures include hydroxybiphenyls, hydroxyacenapthalenes, benzindanols and benztetralinols. Taking into account the uncertainties in mass spectral interpretation, the petroleum and coal liquid are neverless believed to contain all of the possible -14 O isomers, with hydroxybiphenyls being the dominant species. Figure 1A shows evidence for presence of benztetralinols in the Wilmington petroleum ArOH concentrate. Thus, one or both of the acylated parent (M+) -14 O species, represented by ions at m/e 364 and 350, give rise via loss of 42 or 28 mass units respectively to the fragment ion at m/e 322. Although it is possible that the m/e 322 ion is a parent (M+) ion resulting from some abnormally high-boiling -14 O isomer, its production via retro-Diels-Alder loss of propylene or ethylene is much more likely. This fragmentation behavior is characteristic of cyclohexylaromatic systems (25), and thus indicates a benztetralinol structure. The intense ion at m/e 225 in Figure 1A occurs from loss of $CF_3C(O)$+ from the m/e 322 ion; the ion at m/e 344 is a molecular ion from a coeluting -20 O species. Also, this and other spectra in Figure 1 indicate the complexity of spectra from later eluting GC/MS peaks.

-16 O

In the coal liquid and shale oil, this series consists largely of hydroxyfluorenes. In the petroleum, the major structural types are probably phenylindanols/phenyltetralinols. Next to -14 O compounds, -16 O compounds were the second most prevalent type in the SRC-II >325° C ArOH. On the other hand, the -16 O series was a very minor one in the shale oil, and intermediate in the petroleum. The -16 O series was the most condensed ArOH species found in shale oil.

Besides hydroxyfluorenes, the coal liquid contains -16 O compounds which produce retro-Diels-Alder fragmentation patterns similar to those discussed for -14 O compounds. Figure 1B shows an example where the parent (M+) ion at m/e 334 gives rise to a 306 fragment. This pattern is consistent with either a phenyltetralinol or dicycloalkynaphthol(e.g., hexahydropyrenol) structure.

-18 O

Only the >325° C coal liquid ArOH concentrate showed easily detectable amounts of this series. Based on its mass range (Table 2F) and the prevalance of phenanthrenes/anthracenes in coal liquids, this series is believed to be hydroxylated phenanthrenes/anthracenes.

-20 O

This series was found in both the coal liquid and petroleum, but was more prevalent in the coal liquid. Possible structures include phenylnaphthols, cycloalkylphenanthrols and dihydropyrenols. Figures 1 (D-F) show example acylated -20 O series spectra representing the different types present in the coal liquid >325° C residue. Figure 1D shows a fragmentation pattern most likely produced from a C_1-phenylnaphthol. The relatively large M-69 ion (m/e 261) from loss of CF_3+ is also exhibited by hydroxybiphenyls--which would be expected to give spectra analogous to phenylnaphthols. Biphenyls also show a pronounced tendency to form another carbon-carbon bridging bond yielding a m/e 152 ion (26); analogous behavior by phenylnaphthols would yield fluoranthene. Thus, the m/e 218 ion in figure 1D should have a structure analogous to an oxyfluoranthene, and the m/e 202 ion a structure similar to fluoranthene itself.

Figure 1E shows a fragment corresponding to loss of 28 (C_2H_4) from the parent ion at m/e 358. This pattern is typical of a C_1-cyclohexylphenanthrol compound type. No M-69 ion is apparent, but the M-97 (m/e 233) fragment is fairly intense.

Figure 1F shows a large parent ion (m/e 330) relative to fragment ions as well as a long GC retention time relative to its molecular weight. For example, the retention of the compound of equal mass in Figure 1D was only 22.5 min, and the retention of the compound 28 mass units heavier in Figure 1E was only 0.3 min longer than that in Figure 1F. These facts all point to a highly condensed species such as C_1-dihydropyrenol. A dihydropyrene species would be expected to form pyrene (m/e 202) during fragmentation (25), as observed in Figure 1F. Also, Figure 1F correlates closely with Figure 1C, which is believed to be a pyrenol. Both figures show a fragment at m/e 189 corresponding to loss of CO from the m/e 217 fragment. Phenol shows this same fragmentation pattern (27); thus a 1- or 2-pyrenol structure is the most logical for Figure 1C, and a 1- or 2-hydroxy-4,5-dihydromethylpyrene structure is the best choice for Figure 1F. For example, 4-hydroxypyrene or 9-hydroxy-4,5-dihydropyrene would not be expected to give rise to a fragmentation pattern promoting loss of CO to form a cyclopentadienyl-type cation.

Structures for -20 O ArOH in the petroleum could not be defined as well as those from the coal liquid because of coelution of -14 O and -16 O compounds which complicated the resulting spectra.

-22 O, -24 O

As discussed previously, the coal liquid contained -22 O compounds believed to be pyrenols. The possibility of other types of ArOH in this series could not be definitely confirmed or ruled out. -24 O Species in the coal liquid appear to be cycloalkylpyrenols rather than chrysenols or some

other condensed species based on the mass range observed. Again, because of the complexity of the petroleum ArOH spectra, no definite structures for -22 O species were assigned. Based on the mass range of -22 O ArOH in the petroleum, loosely condensed structures such as naphthylindanols, naphthyltetralinols or hydroxytriphenyls seem the most probable.

Non-ArOH species

In contrast to earlier reports citing no or only trace amounts of carboxylic acids in shale oil (14-17), a major series of aliphatic acids ranging from 5 to 27 carbons was observed here. From C_{14} to C_{20}, 2-3 isomers were observed at every carbon number; only one isomer was found at most of the other carbon numbers. Silylated fatty acids yielded characteristic fragment ions at m/e 117, 129, 132, 145, and M-15. During liquid chromatographic separation of the shale oil strong acid fraction, the retention region corresponding to carboxylic acids was not cut as a separate fraction but simply lumped into the ArOH fraction (1). Since previous work had not shown presence of significant amounts of carboxylic acids in shale oil and since their presence in the authors' sample could not be detected by the UV (280nm) detector employed for the HPLC separation, there was no apparent reason for cutting a separate carboxylic acid fraction. Based on total ion intensities of silylated carboxylic acids *vs.* silylated ArOH, carboxylic acids accounted for nearly 50% of the total "ArOH" concentrate. Thus, the result given in Table 1 for concentration of ArOH in shale oil may be high by a factor of two. Figure 2 shows GC/MS total ion chromatrograms for underivatized, acylated and silylated shale oil ArOH concentrate. The later eluting, evenly spaced, peaks in the silylated (2C) sample are all aliphatic acids. Since underivatized carboxylic acids chromatograph poorly, they are not evident in the underivatized or acylated samples. The shale oil investigated in this work was retorted *in situ*; carboxylic acids present in oil shale (28) apparently survived temperatures reached during that type of oil recovery process.

Small amounts of nitrogen compounds (1) and carboxylic acids were present in petroleum ArOH concentrates, largely from chromatographic overlap usually encountered in liquid chromatographic separations. Coal liquid ArOH concentrates were virtually free of non-ArOH compounds.

CONCLUSIONS

The two most distinguishing features of coal liquid ArOH are their high concentration in the whole oil and presence of condensed ring species such as pyrenols and partially hydrogenated pyrenols. Shale oil ArOH are predominantly phenols, some which posses alkyl side chains up to nearly 20 carbons. Petroleum contains relatively low amounts of ArOH but has the greatest diversity of types and isomers present.

The methodology used for analysis of ArOH generally performed well on the wide range of samples examined, except in the case of the 370-535° C petroleum ArOH concentrate. Further improvements including more liquid chromatographic separations, higher resolution GC columns, etc., will be needed for analysis of that sample or one of similar complexity.

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TABLE 1. - Results from distillation and liquid chromatographic separations

Fuel	Distillate Boiling Range (°C)[1]	Distillate Yield (Wt%)	Total Acids (Wt%)		ArOH (Wt%)		
			Distillates Basis	Whole Fuel Basis	Distillate Total Acid Basis	Distillate Basis	Whole Fuel Basis
Coal liquid	200-325	66.3	25.1	16.6	62	15.5	10.3
	325	16.0	30.9	4.94	40.0	12.4	1.98
Shale oil	>200	89.8	16.3	14.6	34.3	5.57	5.00
Petroleum	225-370	25.2	2.24	0.56	5.7	0.13	0.03
	370-535	24.7	10.1	2.49	4	0.4	0.1

[1] Phenol b.p. = 182° C, p-cresol b.p. = 202° C.

TABLE 2A. - Results for -6 O series of ArOH ($C_nH_{2n-6}O$)[1]

		Fuel (Distillate Boiling Range, °C)		
		Coal Liquid (200-325)	Shale Oil (>200)	Petroleum (225-370)
C_x[2]	Molecular Weight	Number of Isomers[3]		
C_0	94	0	1	1
C_1	108	2	3	3
C_2	122	6	6	5
C_3	136	9	11	4
C_4	150	4	12	5
C_5	164	4	12	5
C_6	178	2	9	2
C_7	192		9	1
C_8	206		11	1
C_9	220		7	
C_{10}	234		7	
C_{11}	248		7	
C_{12}	362		4	
C_{13}	376		3	
C_{14}	290		1	
C_{15}	304		2	
C_{16}	318		2	
C_{17}	332		2	

[1] All -6 O compounds were phenols.
[2] C_x = total number of carbons in alkyl substituents.
[3] Number of isomers detected at each alkyl homolog.

TABLE 2B. - Results for -8 O series of ArOH ($C_nH_{2n-8}O$)[1]

		Fuel (Distillate Boiling Range, °C)		
		Coal Liquid (200-325)	Shale Oil (>200)	Petroleum (225-370)
C_x	Molecular Weight	Number of Isomers		
C_0	134	2	2	1
C_1	148	7	6	4
C_2	162	8	10	7
C_3	176	4	11	6
C_4	190	4	12	4
C_5	204		7	1

[1] The dominant structure based on GC/MS fragmentation patterns was indanol for all fuels. Some tetralinols (C_0-tetralinol corresponds to C_1-indanol in mass) were detected in all fuels, however.

TABLE 2C. - Results for -12 O series of ArOH ($C_nH_{2n-10}O$)[1,2]

		Fuel[3] (Distillate Boiling Range, °C)	
		Shale Oil (>200)	Petroleum (225-370)
C_x	Molecular Weight	Number of Isomers	
C_0	144	2	1
C_1	158	4	1
C_2	172	6	2
C_3	186	6	2
C_4	200	2	2
C_5	214	1	

[1] No appreciable amounts of -10 O ArOH were detected in any fuel.
[2] All -12 O compounds were naphthols.
[3] Ions corresponding to naphthols were detected in SRC-II, but they were small in intensity and not definitive enough for a positive identification. A -12 O series corresponding to octahydropyrenols or some similar structure has tentatively been identified in SRC-II >325° C ArOH.

TABLE 2D - Results for -14 O series of ArOH ($C_nH_{2n-14}O$)

		Fuel (Distillate Boiling Range, °C)			
		Coal Liquid		Shale Oil	Petroleum
		(200-325)	(>325)	(>200)	(225-370)
C_x[1]	Molecular Weight				
C_0	170	2	0	2	2
C_1	184	5	10	4	3
C_2	198	2	19	8	5
C_3	212		20	8	10
C_4	226		15	6	12
C_5	240		11	2	11
C_6	254		5	1	8
C_7	268				4
C_8	282				4
C_9	296				2

[1] Unsubstituted hydroxybiphenyls and hydroxyacenaphthalenes have molecular weights of 170. C_0-benzindanols have molecular weights of 184, and C_0-benztetralinols have molecular weights of 198. Hydroxybiphenyls are believed to be the dominant -14 O structure, but all other isomers are also believed to be present.

TABLE 2E. - Results for -16 O series of ArOH ($C_nH_{2n-16}O$)

	Fuel (Distillate Boiling Range, °C)		
	Coal Liquid[1,2] (>325)	Shale Oil[1] (>200)	Petroleum[3] (225-370)
C_x[1] Molecular Weight	Number of Isomers		
C_0 182	4	1	0
C_1 196	12	3	0
C_2 210	19	5	0
C_3 224	19	3	1
C_4 238	15	2	5
C_5 252	6		6
C_6 266	1		4
C_7 280			2
C_8 294			1

[1] Predominantly fluorenols. MW C_0-fluorenol = 182.
[2] Compounds analogous to hexahydropyrenols and phenyltetralinols are also present. MW C_0-hexahydropyrenol = 224.
[3] Based on mass, predominantly phenyltetralinols. MW C_0-phenyltetralinol = 224.

TABLE 2F. - Results for -18 O series of ArOH ($C_nH_{2n-18}O$)[1]

	Fuel (Distillate Boiling Range, °C) Coal Liquid (>325)
C_x Molecular Weight	Number of Isomers
C_0 194	4
C_1 208	5
C_2 222	9
C_3 236	5
C_4 250	3

[1] Presumably hydroxyphenanthrenes/anthracenes. (MW C_0 = 194) A spectrum of a -18 O compound free of other types was not obtained, so positive identification was not possible.

TABLE 2G. - Results for -20 O series of ArOH ($C_nH_{2n-20}O$)

		Fuel (Distillation Boiling Range, °C)	
		Coal Liquid[1] (>325)	Petroleum[2] (225-370)
C_x	Molecular Weight	Number of Isomers	
C_0	220	8	1
C_1	234	20	1
C_2	248	16	4
C_3	262	10	4
C_4	276	3	4
C_5	290	1	1

[1] Dihydropyrenols, phenylnaphthols, and cycloalkylphenanthrols/anthracenols.
[2] MW C_0-dihydropyrenol, C_0-phenylnaphthol and C_0-benzacenaphthalenol = 220. Unknown structure.

TABLE 2H. - Results for -22 O series of ArOH ($C_nH_{2n-22}O$)

		Fuel (Distillation Boiling Range, °C)	
		Coal Liquid[1] (>325)	Petroleum[2] (225-370)
C_x	Molecular Weight	Number of Isomers	
C_0	218	2	0
C_1	232	3	0
C_2	246	2	0
C_3	260	3	2
C_4	274	3	2
C_5	288	1	1

[1] Pyrenols/fluoranthenols. MW C_0 = 218.
[2] Unknown. Possibly naphthylindanols, naphthyltetralinols and/or hydroxytriphenyls. (MW of C_0 homologs = 260, 274, and 246, respectively.)

TABLE 2I. - Results for -24 O series of ArOH ($C_nH_{2n-24}O$)

	Fuel (Distillation Boiling Range, °C)
	Coal Liquid[1] (>325)
C_x Molecular Weight	Number of Isomers
C_0 258	4
C_1 272	3

[1] Probably cycloalkylpyrenols/cycloalkylfluoranthenols. MW C_0-cyclopentylpyrenol = 258. MW C_0-chrysenol = 244 (not observed).

TABLE 3. - Summary of ArOH in the three fuel types

	Fuel (Distillation Boiling Range, °C)				
	Coal Liquid		Shale Oil	Petroleum	
	(200-325)	(>325)	(>200)	(225-370)	(370-535)
Major ArOH compound type Present	phenols	-14 O	phenols	-14 O	Z-series ≥ -16 O
Other important ArOH types	indanols/ tetralinols	-16 O, -20 O, -22 O	indanols/ tetralinols, naphthols	phenols, -16 O, -20 O	
Unique structural features	lack of naphthols	pyrenols and partially hydro-genated pyrenols	long chain C_{10}-C_{17} phenols	cycloalkyl ArOH	cycloalkyl ArOH
Non-ArOH compounds present	none	none	C_5-C_{27} carboxylic acids, indoles	carboxylic acids	carboxylic acids
Overall complexity of concentrate	simple	intermediate	intermediate	complex	very complex

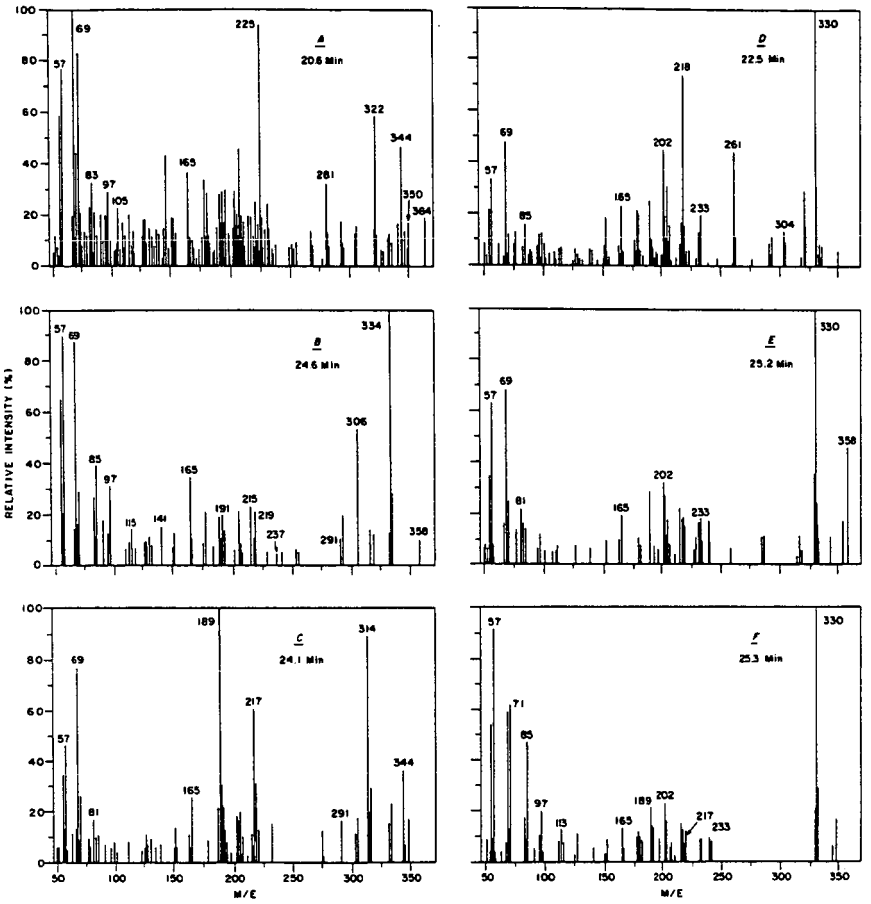

Figure 1 - Mass spectra from GC/MS analysis of acylated Wilmington 225-370° C (A) and SRC-II >325° C (B-F) ArOH concentrates. The GC retention times are noted on each. (A) shows primarily -14 O compounds (m/e 344 is -20 O) (B) shows primarily -16 O (m/e 358 is -20 O) (C) shows -22 O (m/e 314) with minor amounts of other series and (D-F) shows different types of -20 O compounds. For details, see text.

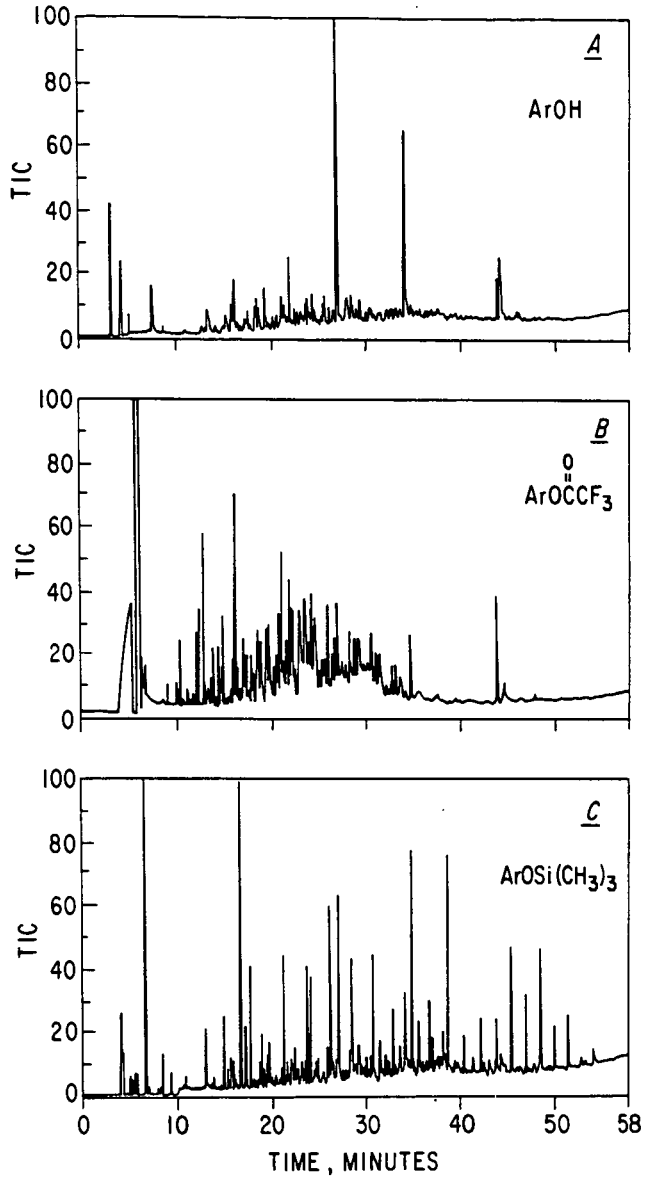

Figure 2 - Total ion GC/MS traces of plain and derivatized >200° C OSCR shale oil ArOH concentrate. Later eluting peaks in (C) are silylated aliphatic acids. Note the high degree of resolution obtained via acylation (B).

Comparative toxicity of crude and refined coal liquids and analogous petroleum products. I. Chronic dermal toxicity in mice.

H. P. Witschi, L. H. Smith, M. E. Goad, M. R. Guerin, W. H. Griest, and C-h. Ho

Biology Division and Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

Introduction

Human exposure to crude and refined coal liquids is most likely to occur via skin contact. In order to estimate eventual risks to human health as a consequence of incidental and prolonged skin contact it is necessary to obtain some information on the potential of coal-derived liquids to elicit skin cancer. This can be done with animals in experimental studies which mimic anticipated human exposure. In addition it also must be established whether prolonged dermal exposure will produce signs of toxicity not only on the skin, but internal organs. This paper reports data obtained in a life-long skin painting study with mice designed to explore these questions.

Materials and Methods

The following materials were tested: Raw H-coal blend, containing 5700 ppm N; H-coal blend after low hydrotreatment (2650 ppm N); H-coal blend after high hydrotreatment (0.2 ppm N); H-coal "home heating oil", a devolatilized version of the high-hydrotreatment H-coal blend; and an H-coal reformed naphtha. Two petroleum-derived reference samples were obtained from the American Petroleum Institute: Petroleum No. 2 fuel oil and high catalytically cracked naphtha (gasoline). Benzo(a)pyrene (99% pure; Sigma Chemical Company) was used as reference substance.

Experimental animals were male and female C3H mice, bred and maintained in the barrier facility of the Biology Division, Oak Ridge National Laboratory. The test agents were applied three times a week onto the shaved skin of the back. The following concentrations were used: 50 ul of undiluted material, 50 ul of a 1:1 or 50 ul of a 1:3 dilution with acetone. Positive controls received 50 ug, 25 ug, or 12.5 ug of benzo(a)pyrene in 50 ul of acetone per application (three times a week) and negative controls were painted with acetone alone or not painted at all. Twenty-five animals of each sex were used per dose level.

All animals had free access to food and water and were kept 5 to a cage on hardwood shavings for the duration of the study. They were weighed periodically. The animals were treated on Mondays, Wednesdays and Fridays. Whenever a skin abnormality became visible at the site of test agent application, painting continued for another two weeks. When the skin mass was found to persist, the animal was killed, and the skin lesion excised and fixed in neutral buffered formalin. A complete necropsy was performed, all observations were noted and selected tissues were fixed in neutral buffered formalin. All skin lesions were examined on paraffin sections under the light microscope. Animals not showing any skin lesions were continuously painted until found moribund or dead. A complete necropsy was performed on all animals killed or found dead.

Results and Discussion

Data on overall tumor incidence are shown in Table 1 and can be summarized as follows: In animals treated with 50 ug of benzo(a)pyrene per application, the first skin lesions began to appear 14 weeks later. In animals given the lowest dose of benzo(a)pyrene (12.5 ug per application) the first tumors appeared after 19 weeks. Tumor incidence then rose dramatically in all animals exposed to benzo(a)pyrene within 5-10 weeks after appearance of the first skin lesion. All animals exposed

eventually developed skin tumors. Histologically, the majority of tumors, 70%, were squamous cell carcinomas. On the other hand, in animals painted with acetone alone or not painted at all, very few skin tumors were observed which were benign papillomas.

Among the coal liquids only the raw H-coal blend produced an almost 100% incidence of skin tumors at all 3 concentrations tested. The first tumors were seen 10 weeks after the first application of 50 ul of the undiluted material (highest dose group). With the lower concentrations, the first tumors were seen after 20 and 33 weeks, respectively. Once the first tumor was discovered in any group, it took approximately one year until most animals in a given group had developed skin tumors. Of these tumors 46% were malignant squamous cell carcinomas.

Hydrotreatment dramatically reduced the carcinogenic potential. The low hydrotreatment preparation, painted undiluted onto the skin, produced the first tumors after 58 weeks, and 92 weeks after beginning of the experiment, tumor incidence was only 14%. In animals exposed to the high hydrotreated preparation, tumors began to appear usually after 70 to 80 weeks and the final tumor incidence in all 3 dose groups was between 16% and 44%. Only 2 tumors were found in the 150 animals exposed to H-coal reformed naphtha. However, in animals exposed to H-coal "home heating oil", the devolatilized version of the highly hydrotreated H-coal blend, tumors began to appear around 40 to 50 weeks of exposure and final tumor incidence in animals exposed to the undiluted material was 30%.

The two petroleum-derived samples had practically no carcinogenic potential; only 3 animals with tumors overall were found in animals painted with gasoline and only a total of 13 animals had tumors 95 weeks after beginning of the experiment. An evaluation of the gross necropsy findings showed that many animals suffered from lesions usually expected to develop in a certain percentage of aging animals, such as myocardial calcification, renal failure, liver tumors, ovarian tumors, and lymphomas, among others. However, the gross lesions were not associated with any particular treatment regimen and were also seen in the control and untreated animals.

It is concluded that the carcinogenic potential of a raw H-coal blend can be mostly abolished by hydrotreatment; however some carcinogenic potential remains associated with a devolatilized version of a severely hydrotreated sample. Petroleum derived products have considerably less carcinogenic activity, an observation compatible with earlier findings. Finally we did not find gross signs of toxicity in organs other than the skin, and the compounds treated seemed not to act as systemic carcinogens.

Acknowledgement

We wish to thank the American Petroleum Institute (API) for having made the two samples available to us.

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Table 1: Skin Tumor Incidence

Compound	Dose per[1] application	No. of animals with skin tumors/ No. of animals exposed	Median time to tumor (days)[2]
931 Raw H-coal blend	50	41/50	45
	25	45/50	52
	12.5	44/50	62
934 Low hydrotreated (2650 ppm N)	50	7/50	112
	25	6/50	117
	12.5	1/50	149
935 High hydrotreated (0.2 ppm N)	50	10/50	100
	25	17/50	111
	12.5	8/50	119
978 H-coal "home heating oil"	50	14/50	97
	25	12/50	108
	12.5	8/50	112
936 H-coal reformed naphtha	50	0/50	-
	25	0/50	-
	12.5	2/50	155
975 API No. 2 fuel oil	50	5/50	120
	25	6/50	131
	12.5	2/50	144
976 API gasoline	50	3/50	125
	25	4/50	130
	12.5	6/50	143
Benzo(a)pyrene[3]	0.1	25/25	21
	0.05	25/25	24
	0.025	25/25	31

[1]High dose was 50 ul of undiluted material; 50 ul lower doses obtained by dilution with acetone; all doses applied 3 times weekly.

[2]Animals killed 2 weeks after appearance of skin tumors; all tumors confirmed by histological diagnosis.

[3]Doses are in percent (w/v) benzo(a)pyrene, 50 uL per mouse.

NON-ADDITIVE MUTAGENIC RESPONSES BY COMPONENTS OF COAL-DERIVED MATERIALS

R. Schoeny,[1] D. Warshawsky,[2] and G. Moore[3]

[1] U.S. EPA, Cincinnati, Ohio 45268
[2] Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, Ohio 45267
[3] U.S. DOE, Pittsburgh Energy Technical Center, Pittsburgh, PA 15236

In the last decade major efforts have been made to ensure the energy self-sufficiency of this country. Included in that effort has been the development and refinement of coal conversion technologies as a means of permitting increased usage of coal resources without attendant increases in pollution. Processes of coal liquefaction and gasification are not, however, free from hazard risk. Solvent refined coal materials SRCI and SRCII, fractions from Bergius and Fisher-Tropsch operations, and H-coal process products have been shown to produce adverse biological effects, such as tumors in experimental animals or mutations in various test system (e.g. 1-3, 7-10). Analytical data indicate that polycyclic aromatic hydrocarbons (PAH), heterocyclic compounds, aromatic amines and other materials with carcinogenic potential are likely to be produced by liquefaction processes (1, 3-6, 16). Coal conversion materials are highly complex and ill-defined mixtures. Coal liquids, for example, have a more complex composition than do crude petroleums of a similar boiling range. Mutagenic or carcinogenic components may, in fact, constitute a very minor percentage of the mass of these materials.

For these, as for other mixtures of biologically active materials, the question has been raised whether the individual activities of constituents are additive or if there are interactive effects contributing to the overall biological activity observed for the mixture. There are a few examples of non-additive biological responses in the literature. It is known, for instance, that mixtures of PAH and aromatic amines showed greater than additive effects when assayed for mutagenicity of Salmonella typhimurium (11). Likewise, a mixture of benzo[a]-pyrene (BaP) dihydrodiols was more directly mutagenic than would be expected based on the mutagenic activities of the individual diols. When rat liver homogenates (S9) were added to assay system, the reverse was true (12). Our laboratory has also observed that fractions of coal-derived materials exhibited non-additive mutagenic responses in Salmonella typhimurium (14). Current research has been directed toward an examination of synergistic or antagonistic biological activities of coal conversion mixture components.

MATERIALS AND METHODS

Samples and Preparation

Samples were supplied through U.S. DOE PETC by R.A. Winschel of the Coal Research Division of Conoco. All materials were collected on a daily basis, combined and distilled by Conoco. Sample PDU-9 is a hydroclone overflow material from an H-coal process design unit. Lummus Feed and Lummus Product are the second stage feed and product, respectively from run 3LCF9 of a Lummus Integrated Two Stage Liquefaction process. Samples were stored in the dark at 5°C. They were prepared for assay by weighing 100-500 mg and adding dimethylsulfoxide (DMSO) so as to obtain a presumptive concentration of 10 or 20 mg/ml. Sample solutions were filter sterilized and applied as 0.1 ml aliquots for reverse mutation assays and as 1 ul aliquots for forward mutation assays. Tared vessels containing undissolved materials were dried and weighed, and this figure used in calculation of the true concentration of the test solutions.

Preparation of Solvent Extracts of Samples

Approximately 5g of each sample was weighed and a volume of solvent equal to 5x of the weight was added in capped tubes. This mixture was agitated vigorously in the dark at room temperature for 2 hrs. After centrifugation to settle particulates, the solvent was removed and an equal amount of fresh solvent was added. The mixture was agitated for another 2 hours followed again by centrifugation. The two solvents with extracted material were pooled and evaporated under N_2. This procedure was carried out sequentially with hexane, toluene, methylene chloride, and acetonitrile. There was generally a quantity of unextracted material remaining, which is referred to in the text and tables as the residue fraction. The DMSO soluble portion of this material and all the dried extracts were prepared for assay in the same manner as the whole sample.

Preparation of "Reconstructed Whole" Samples

"Reconstructed whole" mixtures were prepared by adding individual fractions in the same proportions in which they were extracted. For example, PDU-9 fraction III reconstruction consisted of 1.71% hexane fraction, 42.76% toluene fraction, etc. Materials were dissolved in the original extracting solvents or weighed out, combined, and the solvents evaporated under N_2. The reconstructions were assayed as DMSO solutions on the same occasion as were aliquots of the unfractionated sample and the individual fractions.

Mutagenicity Assays in Salmonella Typhimurium

Liver homogenates (S9) for mammalian metabolism were prepared from male Sprague-Dawley rats which had been injected i.p. with 500 mg/kg Aroclor 1254 and killed five days thereafter. Livers were homogenized in 3 volumes cold buffer (0.15 M KCl, 0.05M Tris HCl). The supernatant fraction from 20 min. centrifugation at 9000g was dispensed into 0.5 ml aliquots and stored at -70°C until use.

Reverse mutation plate incorporation assays using Salmonella typhimurium strains TA97 and TA98 were done according to published procedures (9). All assays included use of 50 ul S9/plate plus appropriate buffers and cofactors.

Strain TM677 was used in forward mutation assays wherein resistance to 8-azaguanine toxicity was measured as the endpoint (15). Fresh cultures (from frozen aliquots) of approximately $3x10^7$ organisms were used in microvolume suspension assays, total volume 100 ul. The test material was added as 1 ul aliquots and S9 comprised 1% of the total assay volume. Duplicate assay tubes per experimental point were incubated for 2 hr at 37°C in a gyrotory bath. At the end of the incubation period cells were diluted 1/5 with phosphate buffered saline and 145 ul plated in triplicate for selection of mutant organisms. An aliquot was further diluted and plated in the absence of selection for determination of bacterial survival. Numbers of revertants/10^5 surviving cells were calculated from the 6 mutagenicity and 6 toxicity plate counts per experimental point. A positive response in this assay is one in which the number of mutants/10^5 survivors is greater than the upper 99% confidence limit on both day of assay and mean historical spontaneous controls.

Results and Discussion

Sample PDU-9 is a vacuum still bottom material from an H-coal process design unit using Kentucky 11 coal and run in the Syncrude mode. The Lummus process which provided the Feed and Product samples consisted of a short contact time liquefaction, followed by antisolvent deashing and subsequent upgrading of

the deashed coal liquids in a Lummus-Cities Service LC fining unit. The Lummus Feed is the LC-Finer feed stock, and Lummus Product the recycle oil produced by this unit.

All materials were assayed for mutagenicity as DMSO solutions. All were mutagenic in both reverse mutation assays to histidine prototrophy (Ames test) and in a forward mutation assay using selection of 8-azaguanine resistant clones. Note that the latter test incorporates a 2-hour period wherein the Salmonella and test items are incubated in liquid suspension. For all assays mutagenicity was detectable only when rat liver homogenates (S9) were included to provide mammalian enzymes for metabolism of the materials to reactive forms.

Of the three samples, PDU-9 was most mutagenic, and is, in fact the most mutagenic fuel material assayed in our laboratory. This sample, and all others, was diluted and assayed at 5 concentrations. The assay was repeated on this set of solutions and then a fresh set of sample solutions was prepared and tested on two occasions. As these coal materials are heterogenous in composition, there are variations both in the amount of material soluble in DMSO and in the mutagenicity observed. Data from assay of PDU-9 graphed in Figure 1, illustrate the variation in mutagenic response. It is also clear from this figure that, at low sample concentrations, there is a linear sample-dose/mutagenic response relationship. It is possible, therefore, to obtain an estimate of the mutagenic response per unit sample by applying regression analysis to those points and obtaining the slope of the regression line. For the PDU-9 data graphed in Figure 1, one obtains an estimate of 50.6 TA98 colonies/ug sample and 48.1 TA97 colonies/ug. Mutagenicity observed for Lummus Feed was 13.4 TA97 and 4.2 TA98 Colonies/ug and for Lummus Product 17.9 TA97 and 11.7 TA98 colonies/ug. Strain TA98 is known to respond to a variety of compounds which cause frameshift mutations, including PAH and aromatic amines. Strain TA97 also is reverted by frameshift mutagens but is particularly sensitive to acridine compounds and probably to other heterocyclics as well (8). The difference in response by the two strains of the Lummus vs the H-coal (PDU-9) samples is suggestive of variations in their composition. It is expected that the Lummus materials will be shown to be composed of a greater percentage acridines and related compounds than the H-coal sample.

The forward mutation assay presumably detects all types of mutagenic events, including deletions and translocations, that are compatible with bacterial viability. The use of a suspension incubation gives the added advantage of providing a relevant quantitation of test item toxicity. None of the test materials when assayed with S9 were toxic to the Salmonella; that is, caused a reduction of more than 40% viability. All three proved to give positive results, as measured by increases in numbers of mutants/10^5 survivor greater than the upper 99% confidence limit on both day-of-assay and mean historical spontaneous controls. Furthermore, the magnitude of the mutagenicity response was seen to increase in a linear fashion with sample concentration, allowing the same sort of estimation as for the reverse assay data. Sample PDU-9 was also the most mutagenic sample as measured in the forward mutation assay, with 2.94 mutants/10^5 survivors/ug compared to Lummus Feed (0.11 mutants/10^5 survivors/ug) and Lummus Product (0.45 mutants/10^5 survivors/ug).

As a first step in the analysis of these fuel materials, a simple fractionation process was undertaken. Sequential organic solvent extracts were prepared using solvents of increasing polarity; namely, hexane, toluene, methylene chloride, and acetonitrile. The DMSO-soluble portion of the non-extractable material (residue) left at the end of the process, and all fractions were assayed for mutagenicity in forward and reverse assays as described for the uncut samples. Two sets of fractions were prepared and assayed.

Generally these materials were similar in their extraction properties to other H-coal vacuum bottoms we have assayed (13, 14, 17). Little material is extracted by hexane and less than 1% of the total mass was acetonitrile extractable. From 20-36% of the material was left at the end of the procedure as the residue fraction. In the course of the fractionation, the sample gains weight, presumably due to retained solvent or water. Use of solvents which had been dried by the addition of a water sieve agent did not reduce this weight gain.

The fractions differ markedly from one another in their mutagenic activity (Tables 1-5). As one would expect, the residue fraction of the extraction process is the least mutagenic material. The components extracted by acetonitrile are generally highly mutagenic (e.g. PDU-9 100,940 TA98 colonies/mg; 73,272 TA97 colonies/mg). This latter fraction, presumably the most polar components of the coal-conversion materials, is present in small amounts. The proportionate activity of the whole mixture is, therefore, very small, assuming additivity. If one multiples the calculated mutagenicity of a fraction in colonies/mg by the per cent of the whole mixture that fraction represents, a determination of fraction specific activity can be made. For example, in Table 1, the TA98 mutagenicity of the residue fraction was low, 2544 colonies/mg, but these components constitute 23.84% of the sample. The specific activity of the residue, or its contribution to the mutagenicity of the whole sample, was 606 colonies/mg whole sample. The highly mutagenic acetonitrile fraction contributed to the same extent to whole sample mutagenicity (596 colonies/mg whole sample) as it constituted only 0.59% of the PDU-9 sample mass.

If there are no interactive effects among fractions of these coal-conversion materials, the sum of the specific activities should equal the mutagenicity observed for the unfractionated material. When these summations have been calculated for a variety of coal-conversion samples the percentages have been found to be comparable to the whole sample mutagenicity. There have also been observed sums of fractional activities less than the whole as well as greater than the whole (14). PDU-9, Lummus Product and Lummus Feed fall into the former category. This non additivity of fraction mutagenic activities is unlikely to be due to dilution as a result of weight increase of the material during the extraction process. In no instance was the weight gain more than 20% of the original sample weight (PDU-9). Table 1 indicates that the "loss" of PDU-9 mutagenicity with fractionation was about 50% for the reverse mutation assay (Table 2). The data for Lummus Feed are similar (Tables 4,5), whereas dilution cannot be discounted as accounting for the majority of the non-additivity observed with the Lummus Product sample (Table 3).

Another explanation for non-additivity is the loss or alteration of mutagenic compounds as a consequence of the extraction process. It was believed that this mild extraction, which entails no changes in pH or temperature of the material, would be unlikely to generate oxygenated or otherwise modified compounds. The protocol was undertaken under yellow lights to minimize photoreactions of PAHs and other aromatic compounds. A third possibility is that the fractional components act as co-mutagens.

To elucidate the mechanism of the observed non-additivity, "reconstructed whole" mixtures were prepared. This was done by recombining the sample fractions in the same proportions which they were extracted. The reconstructions were assayed in reverse and forward mutation assays on the same occasions as the unfractionated whole and the individual fractions. If there are, in fact, co-mutagenic interactions among fractions, the mutagenicity of the reconstructed mixture should be increased relative to the fraction sums. For PDU-9 this proved not to be the case (Table 1). For both strains TA97 and TA98, the reconstruction

mutagenicity was not increased,but was roughly equivalent to the fraction sum. This was also the case for forward mutation assay of the reconstructed sample (Table 2). Data derived from assay of the set B fractions corroborate these results (not shown). The Lummus Product reconstruction assay showed only a very little increase in TA97 mutagenicity. For both of these samples it would appear that non-additivity of organic fraction mutagenicity is due to artifacts of extract preparation.

This does not appear to be the case for the Lummus Feed material. Data from reverse mutation assay of both sets of fractions (Tables 4,5) indicate an increase in mutagenesis on the order of 28% to 65% relative to the fraction sums. That there were alterations in the fractions as artifacts of preparation cannot be discounted, but it is also clear that there is evidence of co-mutagenicity among fractions of the Lummus Feed sample. It should be noted parenthetically that the data in these tables also illustrate the potential for loss or change of biological activity that occurs in these complex mixtures as a function of time. Fraction sets A & B were prepared in assayed over a period of several months.

The premise that single mixtures compounds behave independently or additively when mixed is a subject of continuing discussion. The work described here indicates that complex mixtures of compounds may not have biological activity which can be estimated on the basis of summation of activities of known components. It also points out, however, the difficulty of separating mixture components in a way which leaves them unchanged.

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Table 1

COMPARISON OF PDU-9 FRACTION (SET A) AND RECONSTRUCTED MIXTURE MUTAGENICITIES: REVERSE MUTATION PLATE INCORPORATION ASSAYS

SAMPLE	PERCENT EXTRACTION	COLONIES/mg[a]		SPECIFIC ACTIVITY COLONIES/mg[b]	
		TA97	TA98	TA97	TA98
Hexane	1.71	16,850	18,881	288	323
Toluene	42.76	9,516	15,903	4,069	6,800
Methylene Chloride	31.11	29,319	49,017	9,121	15,249
Acetonitrile	0.59	73,272	100,940	432	596
Residue	23.84	1,404	2,544	335	606
Whole				28,056	49,092
Sum of Fractions				14,245 (50.8%)	23,574 (48.0%)
Reconstruction				15,829 (56.4%)	16,389 (33.4%)

[a] Calculated from linear portions of dose response curves
[b] Colonies/mg x per cent extraction

Table 2

COMPARISON OF PDU-9 FRACTION (SET A) AND RECONSTRUCTED MIXTURE MUTAGENICITIES: FORWARD MUTATION ASSAY

SAMPLE	PERCENT EXTRACTION	TM677 COLONIES/ 10 SURVIVORS/mg	SPECIFIC ACTIVITY COLONIES/10^5 SURVIVORS/mg
Hexane	1.71	187	3
Toluene	42.76	405	173
Methylene Chloride	31.11	1,084	337
Acetonitrile	0.59	2,514	15
Residue	23.84	57	14
Whole			2,911
Sum of Fractions			542 (18.6%)
Reconstruction			313 (10.8%)

[a] Calculated from linear portion of dose response curves
[b] Colonies/10^5 survivors/mg x per cent extracted

Table 3

COMPARISON OF LUMMUS PRODUCT FRACTION (SET A) AND RECONSTRUCTED MIXTURE MUTAGENICITIES: REVERSE MUTATION PLATE INCORPORATION ASSAYS

SAMPLE	PERCENT EXTRACTED	COLONIES/mg[a]		SPECIFIC ACTIVITY COLONIES/mg[b]	
		TA97	TA98	TA97	TA98
Hexane	4.76	31,373	6,309	1,493	300
Toluene	26.39	13,128	11,880	3,463	3,135
Methylene Chloride	16.50	28,341	38,141	4,676	6,293
Acetonitrile	1.26	18,862	23,775	238	300
Residue	51.08	2,545	4,686	1,300	2,394
Whole				15,730	21,844
Sum of Fractions				11,170 (71.0%)	12,422 (56.9%)
Reconstruction				12,718 (80.9%)	13,482 (61.7%)

[a] Calculated from linear portion of dose response curves
[b] Colonies/mg x per cent extracted

Table 4

COMPARISON OF LUMMUS FEED FRACTION AND RECONSTRUCTED MIXTURE MUTAGENICITIES: REVERSE MUTATION PLATE INCORPORATION ASSAYS, STRAIN TA97

SAMPLE	PER CENT EXTRACTED		TA97 COLONIES/mg[b]		SPECIFIC ACTIVITY TA97 COLONIES/mg[c]	
	A	B	A	B	A	B
Hexane	2.48	2.13	8,862	376	220	8
Toluene	25.94	17.88	9,603	8,721	2,491	1,559
Methylene Chloride	34,28	37.76	15,106	10,200	5,178	3,852
Acetonitrile	1.06	0.75	22,265	28,732	236	215
Residue	36.24	41.48	2,540	726	920	301
Whole					17,952	13,411
Sum of Fractions					9,045 (50.4%)	5,935 (44.2%)
Reconstruction					15,385 (85.7%)	12,162 (90.7%)

[a] Refers to fraction set A or B
[b] Calculated from linear portions of dose response curves
[c] Colonies/mg x per cent extracter

Table 5

COMPARISON OF LUMMUS FEED FRACTION AND RECONSTRUCTED MIXTURE MUTAGENICITIES: REVERSE MUTATION PLATE INCORPORATION ASSAYS, STRAIN TA98

SAMPLE	PER CENT EXTRACTED		TA98 COLONIES/mg[b]		SPECIFIC ACTIVITY TA98 COLONIES/mg[c]	
	A[a]	B	A	B	A	B
Hexane	2.48	2.13	4,376	458	108	10
Toluene	25.94	17.88	4,876	3,454	1,265	618
Methylene Chloride	34.28	37.76	4,436	3,973	1,521	1,500
Acetonitrile	1.06	0.75	20,630	10,192	902	151
Residue	36.24	41.48	2,490	364		
Whole					11,127	5,002
Sum of Fractions					4,015 (36.1%)	2,355 (47.1%)
Reconstruction					7,183 (64.6%)	5,643 (112.8%)

[a] Refers to fraction set A and B
[b] Calculated from linear portion of dose response curves
[c] Colonies/mg x per cent extracted

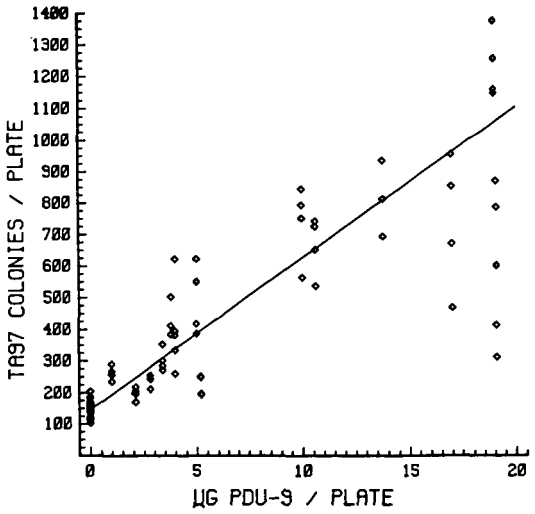

Figure 1. Mutagenicity of coal hydrogenation sample PDU-9 for Salmonella typhimurium strain TA98. The solid lines the least squares line for these data, n = 97, r = 0.9259.

IDENTIFICATION AND MUTAGENICITY OF AMINO- AND HYDROXY-SUBSTITUTED NITROGEN AND SULFUR HETEROCYCLES IN A SOLVENT-REFINED COAL LIQUID

Masaharu Nishioka, Gary M. Booth, and Milton L. Lee

Departments of Chemistry and Zoology
Brigham Young University
Provo, UT 84602

Raymond, N. Castle

Department of Chemistry
University of South Florida
Tampa, FL 33620

INTRODUCTION

Development of coal upgrading processes such as gasification and liquefaction must be continued because of the long-term uncertainty in the availability of petroleum resources. Identification of components and investigation of the environmental effects of coal-derived products produced in these upgrading processes are required. Although numerous studies describing the identification and characterization of polycyclic aromatic hydrocarbons (PAH) and polycyclic aromatic compounds (PAC) containing a single heteroatom have been reported, PAC containing more than one heteroatom have been only tentatively identified until recently. In the last year, many new PAC containing two heteroatoms in a solvent-refined coal liquid were positively identified using capillary column gas chromatography. These compounds include hydroxylated thiophenic compounds (1), aminodibenzothiophenes (2), hydroxylated nitrogen heterocycles (3), azathiophenic compounds (4), and amino-substituted nitrogen heterocycles (5). In this paper, the analytical methods for the identification of trace amounts of PAC containing two heteroatoms in complex coal-derived materials are reviewed. The structural characteristics of the PAC formed in a solvent-refined coal liquid and a coal tar were compared, and standard compounds were synthesized for verification of identifications and for mutagenicity testing.

EXPERIMENTAL

A solvent-refined coal heavy distillate (SRC II HD: 260-450°C boiling point range) was obtained from the Fort Lewis, Washington, pilot plant (operated by the Pittsburg & Midway Coal Mining Co.). A coal tar was obtained from the National Bureau of Standards, Washington, DC. This was a medium crude coke oven tar. Standard compounds were obtained commercially or synthesized by us (6).

The SRC II HD material and coal tar were fractionated into chemical classes by adsorption chromatography on neutral alumina and silicic acid (3, 7). The third (A-3) and fourth (A-4) alumina column fractions which were composed of the nitrogen- and hydroxy-containing PAC, respectively; the second (S-2) and third (S-3) silicic acid column fractions from the A-3 fraction which were composed of the amino- and tertiary nitrogen-containing PAC, respectively; and the second (S-2′) silicic acid column fraction from the A-4 fraction which was composed of hydroxy nitrogen-containing PAC were analyzed in this study.

Hewlett-Packard Model 5880 gas chromatographs equipped with flame ionization (FID), sulfur-selective flame photometric (FPD), nitrogen-selective thermionic (NPD), and ^{63}Ni electron capture (ECD) detectors were used in this study. The capillary columns were prepared by coating 10-20 m x 0.2 or 0.3 mm i.d. fused silica tubing (Hewlett-Packard, Avondale, PA) with SE-54, a 25% biphenyl polymethylsiloxane (8,9), or a smectic liquid-crystalline polymethylsiloxane stationary phase (10-13) (0.25 μm film thicknesses). A Hewlett Packard Model 5982A GC-MS system was used to obtain mass spectral confirmation of identified compounds. The Ames mutagenicity assays were performed as described by Ames et al. (14) with minor modifications.

RESULTS AND DISCUSSION

The chemical complexities typically associated with coal-derived products require stationary phase selectivity in addition to high efficiency for their analysis by capillary column gas chromatography. We have recently synthesized a new polarizable stationary phase, a biphenyl polysiloxane (8,9), and several liquid-crystalline polysiloxanes (10-13) for the analysis of PAC. The biphenyl polysiloxane provided unique selectivity for the polar amino- and hydroxy-substituted nitrogen and sulfur heterocycles. The polar substituent groups on the PAC interact with the π-electron cloud of the biphenyl group. Blocking of polar groups is sometimes recommended for strongly polar compounds. Blocking of the polar group leads to a decrease in the dipole-induced dipole interactions between solutes and the stationary phase. This "softer" interaction often results in improved resolution. All isomers of the hydroxydibenzothiophenes in the coal tar were resolved and identified using a 25% substituted biphenyl phase (Figure 1) after blocking of the hydroxyl group. In comparison, only the 1- and 2-hydroxydibenzothiophenes were detected in the SRC II HD. Similarly, improved resolution was observed for the pentafluoropropyl (PFP) derivatives of the alkylated aminodibenzothiophenes (2). The large retention differences between the original polar compounds and their derivatives gave complementary evidence for their identification (1,2).

The separations achieved on liquid-crystalline stationary phases is based on the molecular geometries of solutes, such as size, shape, and planarity, in addition to other factors associated with conventional stationary phases. Positive identifications of the hydroxylated thiophenic compounds were achieved using both a smectic liquid-crystalline stationary phase and the 25% biphenyl stationary phase (1). This liquid-crystalline phase was also used for the separation of isomeric PAH (15), sulfur heterocycles (16), and amino-PAH (5).

Approximate concentrations of the amino- and hydroxy-substituted nitrogen and sulfur heterocycles were estimated to be 1-10 ppm in the SRC II HD. In comparison, corresponding PAC containing only one heteroatom were present at approximately 100-1000 ppm, and PAH were found to be present at 0.1-5% in the same sample (17). The amino- and hydroxy-substituted heterocycles were identified at low levels by utilizing selective detectors during the analysis of fractions in which one of the two functional groups was enriched: FPD for the nitrogen heterocycles and amino-PAC fraction, FPD for the hydroxyl-PAC fractions, ECD for the tertiary nitrogen PAC fraction, and NPD for the hydroxyl and nitrogen-PAC fractions. Compounds were positively identified by comparison of retention data with those of standard compounds and by selected ion mass spectrometry. Figure 2 shows one example: the ECD chromatogram of the PFP-derivatized SRC II HD S-3 fraction. This chromatogram shows only the

PFP-amide and tertiary nitrogen-containing compounds; the aminophenylpyridines, aminophenylquinolines, and their alkylated derivatives were identified by GC-MS.

All isomers of the aminodibenzothiophenes and the azadibenzothiophenes were assayed for mutagenicity using the Ames test (2,4). The 2- and 3-aminodibenzothiophenes were strongly mutagenic, and their average response was ten to one hundred times greater than the average response of benzo[a]pyrene, while all isomers of the azadibenzothiophenes were inactive. Recently, the microbial mutagenicities of numerous isomeric 3- to 5-ring sulfur heterocycles have been extensively studied (18-20). The presence of a sulfur heteroatom was found to have little effect on mutagenicity. Likewise, compounds containing both nitrogen and sulfur heteroatoms in the rings also demonstrated little mutagenic activity.

Representative PAC structures found in the SRC II HD and the coal tar containing one heteroatom and two heteroatoms are given in Tables 1 and 2, respectively. It is thought that the major reason for the difference between the two samples was auto-catalytic mild hydrogenation in the SRC II process. This would explain the difference in abundance of the amino compounds, hydroxyl PAC, sulfur heterocycles with fusion on only one side of the thiophene ring, 4H-benzo[def]carbazole, etc. The amino and hydroxyl groups in the SRC II HD are thought to be derived from nitrogen and oxygen heterocycles by hydrogenation. The 2- and 3-ring amino-PAH in the SRC II HD were the major components of the amino-PAH fraction, while only the aminonaphthalenes were detected in the coal tar, and then only at a low level (5). Likewise, the aminodibenzothiophenes in the SRC II HD were present as major components, but only the azathiophenic compounds were present in the coal tar (4). Also, the hydroxyphenylthiophenes and the hydroxyphenylbenzothiophenes were more abundant than the hydroxybenzothiophenes and hydroxydibenzothiophenes in the SRC II HD (1). On the other hand, only the hydroxybenzothiophenes and hydroxydibenzothiophenes were present in the coal tar. Figures 3 and 4 show chromatograms of the hydroxyl nitrogen-PAC fractions of both samples. Marked abundances of the hydrogenated compounds, i.e. hydroxyphenylpyridines, was noticeable in the SRC II HD.

Structural similarities between the heteroatom-containing PAC and the PAH were found. Structures of the PAC containing two heteroatoms were similar to those of the PAC containing one heteroatom, and the structures of the PAC containing one heteroatom reflect the parent PAH structures. The relationships between structure and abundance for these compounds are discussed in detail elsewhere (17).

ACKNOWLEDGMENT

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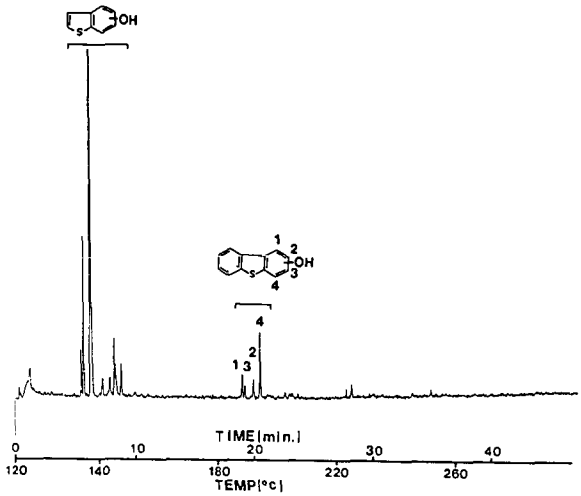

Figure 1. FPD chromatogram of the coal tar A-4 trimethylsilyl derivatized fraction on a 25% biphenyl polysiloxane stationary phase. Conditions: temperature program from 120°C to 265°C at 4°C min^{-1}, after an initial 2-min isothermal period; hydrogen carrier gas at 100 cm s^{-1}.

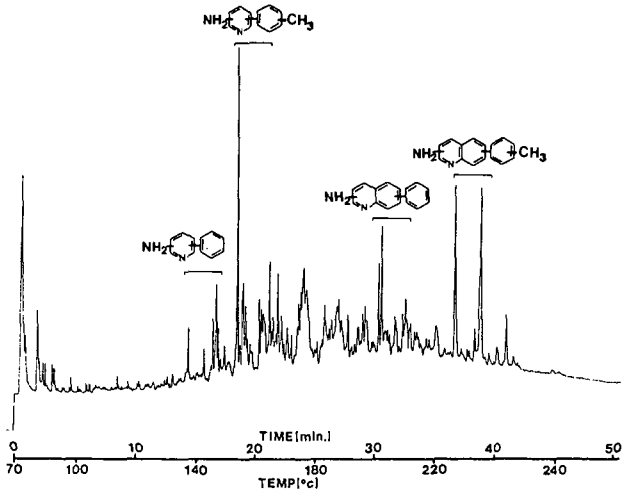

Figure 2. ECD chromatogram of the SRC II HD S-3 PFP derivatized fraction on SE-54. Conditions: temperature program from 70°C to 100°C at 10°C min^{-1}, then from 100°C to 265°C at 4°C min^{-1}, after an initial 2-min isothermal period; helium carrier gas at 50 cm s^{-1}.

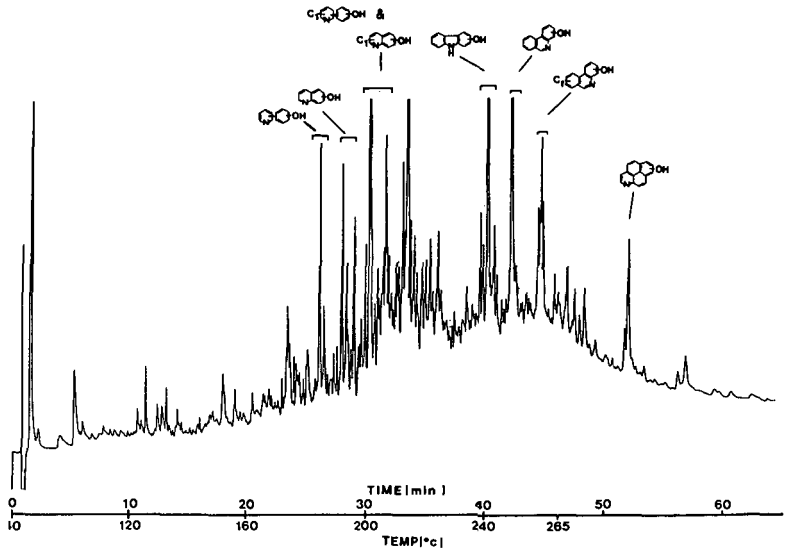

Figure 3. NPD chromatogram of the SRC II HD S-2′ fraction on a 25% biphenyl polysiloxane stationary phase. Conditions: temperature program from 40°C to 120°C at 10°C min^{-1}, then from 120°C to 265°C at 4°C min^{-1}, after an initial 2-min isothermal period; helium carrier gas at 50 cm s^{-1}.

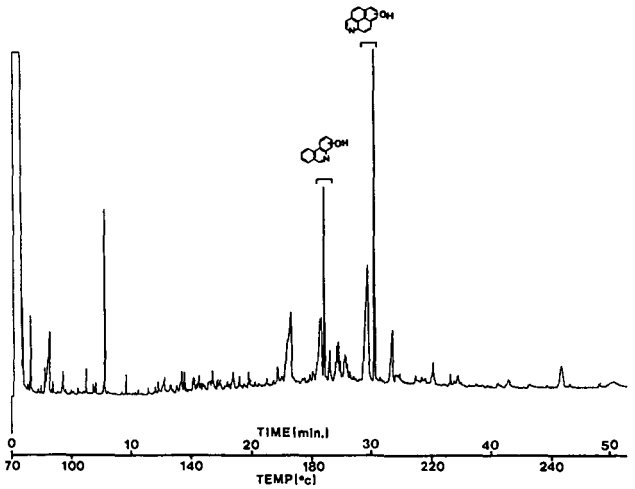

Figure 4. FID chromatogram of the coal tar S-2′ fraction on SE-54. Conditions: temperature program from 70°C to 100°C at 10°C min^{-1}, then from 100°C to 265°C at 4°C min^{-1}, after an initial 2-min isothermal period; hydrogen carrier gas at 100 cm s^{-1}.

Table 1. Comparison of the PAC containing one heteroatom identified in the SRC II coal liquid and the coal tar.

PASH		N-PAC						HPAH	
		2'-PANH		APAH		3'-PANH			
Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II
	–		–	–			–	–	
				–				–	
			–	–			–		–
			–						–
	–		–				–		
									
			–						

Table 2. Comparison of the PAC containing two heteroatoms identified in the the SRC II coal liquid and the coal tar.

PANSH / APASH		HPASH		HPANH	
Coal tar	SRC II	Coal tar	SRC II	Coal tar	SRC II
	–				
		–		–	
–					
	–	–			

Classes of Compounds Responsible for Mutagenic and Cytotoxic Activity in Tars and Oils Formed During Low BTU Gasification of Coal

R.F. Henderson, W.E. Bechtold, J.M. Benson, G.J. Newton, R.L. Hanson, A.L. Brooks, J.S. Dutcher, R.E. Royer, and C.H. Hobbs

Lovelace Inhalation Toxicology Research Institute
P.O. Box 5890, Albuquerque, NM 87185

Summary

The Lovelace Inhalation Toxicology Research Institute (ITRI), in cooperation with the Morgantown Energy Technology Center (METC), has completed toxicity screening of vapors, liquids and solids formed during operation of an experimental pressurized, stirred-bed, coal gasifier at METC. Vapors collected from the cooled process stream on Tenax resins had no mutagenic activity in the Ames Salmonella assay. Dichloromethane extracts of liquids and solids collected from the effluent or process streams were fractionated by gel chromatography into fractions containing mostly aliphatic compounds; neutral polycyclic aromatic hydrocarbons (PAH); polar PAH and heterocyclic compounds; and salts. The polar fraction was partitioned into acids, bases, water soluble compounds and phenols. Bacterial mutagenic activity was highest in the basic fraction with additional activity in the neutral PAHs. Highest cytotoxicity toward both the bacteria and canine alveolar macrophages was in the phenolic fraction. Treatment of the gasifier tars by nitrosation or by acetylation to remove primary aromatic amines (PAA) reduced the bacterial mutagenicity by 50-60%, indicating that some, but not all, of the mutagenicity was due to PAA.

Introduction

The Lovelace Inhalation Toxicology Research Institute (ITRI), working in cooperation with the Morgantown Energy Technology Center (METC), has completed studies to obtain information on the possible inhalation toxicity of airborne effluents associated with low BTU coal gasification (1-4). Such information is needed to enable an improved assessment of potential health risks to man arising from this technology.

The METC coal gasifier is an experimental pressurized, stirred-bed coal gasifier (Figure 1) and differs from commercial fixed-bed producers in its smaller size (1.1 m ID) and its provisions for stirring the bed. The gasifier uses a Lurgi process for low BTU coal gasification using heat, air, steam and coal. The gas cleanup devices are experimental and evolving and are designed to produce a low BTU gas suitable for use in combined cycles with turbines.

The main process stream cleanup devices in use at the time of this research program included a cyclone to remove dust; a humidifier, tar trap and Venturi scrubber to remove tar; a muffler and a flare. Other cleanup devices indicated in Figure 1 were bypassed during sampling periods for this project.

Experimental

Sampling

Vapors, liquids, and solids were sampled from both the process and effluent streams. The process stream was sampled at points A, B, C, D, and E (Figure 1) using two sampling systems. An analytical system extracted cooled, diluted process stream material and measured the concentration (by filters), the size of aerosols (by cascade impactors) and the concentration of vapors (by adsorption on Tenax traps). Condensor traps were used to collect larger samples of tars and oils. In addition to the process stream material, bulk quantities of bottom ash from the gasifier, dust from the cyclone, and tar from the humidifier, tar trap and Venturi scrubber were collected.

Fractionation of Tars and Oils

Tars from the tar scrubbing devices and condensed oils from the process stream were fractionated on Sephadex LH-20 gel columns using tetrahydrofuran (THF) to elute separate fractions containing, 1) mainly aliphatic and polymeric material (F1, F2); 2) neutral polyaromatic hydrocarbons (PAH) (F3, F4); and 3) polar compounds including nitrogen heterocyclic compounds and PAH with polar fractional groups (F5) (See Figure 2). The polar fraction was subfractionated into acidic, basic and neutral components.

Mutagenicity Testing

The potential mutagenicity of each subfraction was assessed using the Ames Salmonella bacterial mutagenicity assay, using strain TA-98 (detects frame-shift mutations) both with and without addition of liver metabolizing enzymes (S-9). Cytotoxicity toward the bacterial cells and toward canine alveolar macrophages was also measured.

Effect of Removal of Primary Aromatic Amines (PAA) on Mutagenicity

To determine the contribution of PAA to the mutagenic activity of gasifier tar, the PAA were removed by nitrosation at pH 2.5 or by acetylation. Several PAA, one aza-arene and a coal oil sample from the Fossil Fuels Research Matrix Porgram, Oak Ridge National Laboratory, were included as control samples. The treated samples were then re-tested for mutagenic activity.

Results

The vapor phase material collected on Tenax traps did not have mutagenic activity in the bacterial mutagenicity assay used. All tar and oil samples collected from the process or potential effluent streams had mutagenic activity when S-9 metabolizing enzymes were included. The subfractions showing the most activity were the neutral PAH (F3, F4) and the polar fraction (F5) in both process stream samples and the potential effluent material (Tables 1, 2, 3). The basic and neutral portions of the polar fraction had

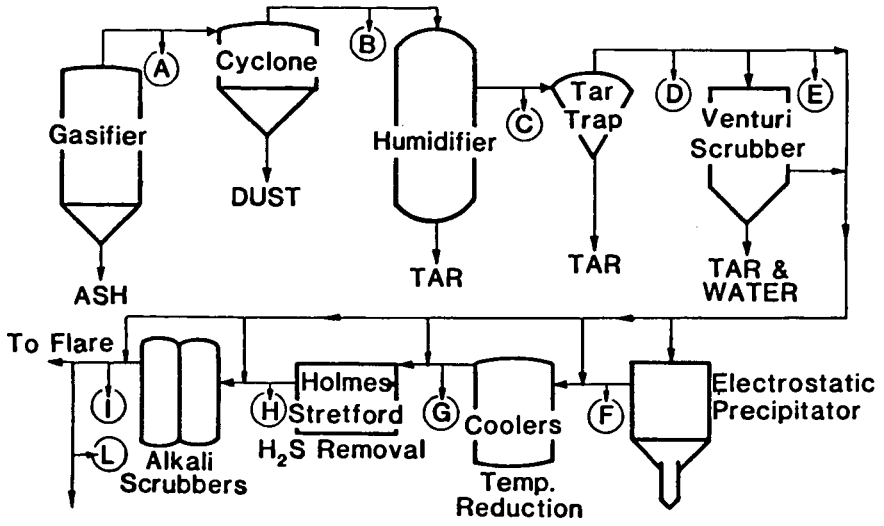

Figure 1. Schematic diagram of the METC low Btu coal gasifier and cleanup system.

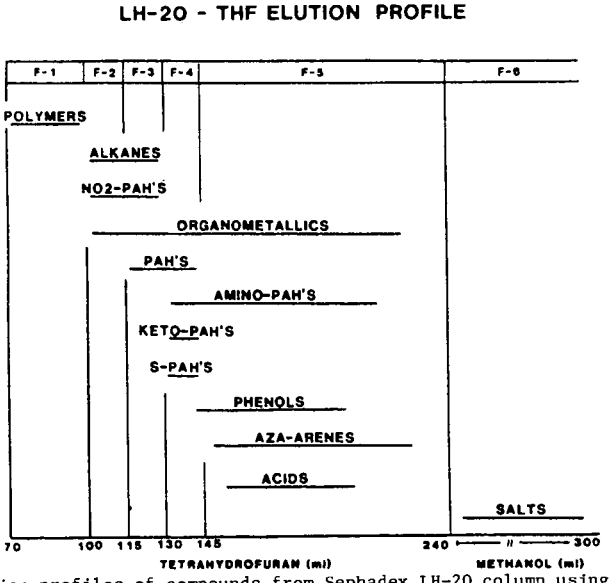

Figure 2. Elution profiles of compounds from Sephadex LH-20 column using tetrahydrofuran (THF) as eluant. The lines for each compound indicate the volume of THF in which the compounds eluted. The dot indicates the center of the elution peak.

TABLE 1

Mutagenic Activity of Process Stream Samples
and their Sephadex LH-20 Fractions

Fraction	Mass[a] Percent	Revertants[b] per μg (with S-9)	Mutagenicity[c] Percent	Revertants per L Process Stream
Position B -- Raw Gas				
Crude	100.0	6.7 ± 0.6	100	50,000
LH-20 Fraction				
1	27.8	0.7 ± 0.2	3	1,600
2	6.5	2.8 ± 0.3	3	1,900
3	21.8	10.4 ± 1.1	39	17,000
4	16.7	5.0 ± 0.6	14	6,200
5	21.7	10.4 ± 1.0	38	17,000
6	5.5	2.8 ± 0.2	3	1,100
Position D -- After Tar Trap				
Crude	100.0	3.7 ± 0.2	100	9,700
LH-20 Fraction				
1	35.4	0.0	0	0
2	8.4	3.2 ± 0.4	14	700
3	18.6	1.9 ± 0.6	18	900
4	15.6	4.5 ± 0.4	35	1,900
5	15.3	4.1 ± 0.2	31	1,600
6	6.7	0.5 ± 0.3	2	80
Position E -- After Venturi Scrubber				
Crude	100.0	4.1 ± 0.3	100	2,200
LH-20 Fraction				
1	7.0	0.2 ± 0.2	0	10
2	5.0	2.8 ± 0.4	6	70
3	23.0	1.8 ± 0.3	24	200
4	14.0	2.3 ± 0.4	18	200
5	29.0	2.9 ± 0.2	49	400
6	21.0	0.2 ± 0.2	3	25

a Mass percent of material fractionated.
b TA-98 revertants/μg determined from slope of dose-response curve by linear regression analysis.
c Mutagenicity percent is the percent of the mutagenicity each fraction contributes to the crude material.

TABLE 2

Mutagenic Activity of Process Stream LH-20
Fractions 5 and their Subfractions

Fraction	Mass[a] Percent	Revertants[b] per μg with S-9	Mutagenicity[c] Percent
Position B -- Raw Gas			
LH-20 Fraction 5	21.7	9.7 ± 0.5	100
Acids	0.9	1.0 ± 0.5	1
Bases	4.1	5.5 ± 1.0	20
Neutrals	1.6	46.7 ± 2.6	79
Amphoterics - Water solubles	15.2	Not tested	-
Position D -- After Tar Trap			
LH-20 Fraction 5	15.3	2.1 ± 0.2	100
Acids	0.03	0.4 ± 0.1	0
Bases	3.8	4.2 ± 0.4	50
Neutrals	4.6	5.2 ± 0.3	50
Amphoterics - Water solubles	6.6	Not tested	-
Position E -- After Venturi Scrubber			
LH-20 Fraction 5	29.0	2.3 ± 0.3	100
Acids	0.7	0.0	0
Bases	1.6	12.4 ± 0.7	83
Neutrals	2.1	2.2 ± 0.2	17
Amphoterics - Water solubles	24.6	Not tested	-

a Mass percent of material fractionated.
b TA-98 revertants/μg determined from slope of dose-response curve by linear regression analysis.
c Mutagenicity percent is the percent of the mutagenicity each fraction contributes to the crude material.

TABLE 3

Mutagenic Activity of Tar Trap Tar and Venturi Scrubber Water and their LH-20 Fractions

Fraction	Mass[a] Percent	TA-98 Revertants[b] per μg (with S-9)	Mutagenicity[c] Percent
Position B -- Raw Gas			
Tar Trap Tar	100	21.6 ± 1.8	100
LH-20 Fraction			
1	16	2.9 ± 0.2	2
2	18	2.9 ± 0.5	2
3	34	2.5 ± 0.8	3
4	14	107.0 ± 49.1	60
5	14	56.6 ± 8.0	32
6	3	10.2 ± 0.5	1
Venturi Scrubber Inlet Water Lyophilized (50ml)	100	0.0	0
Venturi Scrubber Outlet Water Lyophilized (50ml)	100	1.14 ± 0.06	100
Outlet Water Dicholormethane-Solubles (0.07%)	100	0.72 ± (0.18)	100
LH-20 Fraction			
1	6	0.8 ± 0.2	7
2	2	1.1 ± 0.3	3
3	3	1.7 ± 0.3	7
4	5	1.8 ± 0.4	13
5	75	0.6 ± 0.2	64
6	10	0.4 ± 0.2	6

[a] Mass percent of material fractionated.
[b] TA-98 revertants/μg determined from slope of dose-response curve by linear regression analysis.
[c] Mutagenicity percent is the percent of the mutagenicity each subfraction contributes to the total.

the greatest mutagenicity (Table 2). Nitrosation or acetylation of the tar-trap tar removed some (50-60%) of the mutagenic activity (Table 4) but not as much as was removed by similar treatment of a coal oil.

The most cytotoxic fractions of the coals and tars were the polar fractions containing phenols.

Discussion

Tars and oils produced during a low BTU coal gasification process were mutagenic toward Salmonella bacteria. The mutagenic activity could be attributed to PAH and to neutal and basic compounds in the polar fraction. In contrast to coal liquids, in which most of the mutagenic activity has been attributed to PAA (5), the mutagenic activity of the tars was reduced by only approximately one-half after treatment to remove PAA.

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TABLE 4

Mutagenic Activity Remaining After Treatment
(%)

Chemical	Nitrosation	Acetylation
2-Aminoanthracene	4	7
3-Aminofluoranthene	0	20
2-Aminofluorene	0	21
6-Aminochrysene	13	6
9-Aminophenanthrene	0	0
Phenathridine	100	86
Coal oil A[a]	9	30
Tar trap tar	39	52

[a] Obtained as a comparative research material from the Fossil Fuels Research Matrix Program, Oak Ridge National Laboratory.

CHEMICAL BASIS FOR PHOTOMUTAGENICITY IN SYNTHETIC FUELS

Christopher P. Selby[1,2], John Calkins[1,2], Harry G. Enoch[2,3], Cherylyn W. Wright[4] and Bary W. Wilson[4]

[1]Department of Radiation Medicine
and
[2]Graduate Center for Toxicology,
University of Kentucky, Lexington, KY 40536;
[3]Kentucky Center for Energy Research,
P. O. Box 11888, Lexington, KY 40578;
[4]Biology and Chemistry Department,
Pacific Northwest Laboratory,
Richland, WA 99352.

ABSTRACT

Photomutagens (chemicals that enhance the mutagenicity of near UV-visible radiation) have been detected in a variety of experimental coal- and oil shale-derived synthetic fuels using S.typhimurium strain TA98 and fluorescent light. In this study, photomutagenic activity was measured among synfuel samples that included crude and hydrotreated shale oil, coal oil distillation fractions, and chemical class fractions of coal and shale oils. Photomutagenic activity was found to increase with increasing boiling point and was concentrated in fractions enriched in neutral polycyclic aromatic hydrocarbons (PAH). These results point to high molecular weight neutral PAH constituents as important photomutagenic components among the samples tested. The photomutagenic activities of the materials tested correlate well with the previously reported tumorigenic activities of the same samples on mouse skin but correlate poorly with the previously reported mutagenic activities in the conventional Salmonella/mammalian-microsome mutagenicity test in which neutral PAH fractions were inactive.

INTRODUCTION

A need currently exists for developing alternative energy technologies such as producing petroleum substitutes from coal and oil shale. However, the elevated risk for skin cancers seen among past coal and shale oil production and maintenance workers (1,2) points to a potential problem that must be addressed by the developing synthetic fuels technologies. The hazards associated with synfuel processes and materials should be identified and minimized prior to commercial production. Accurate toxicological data is, therefore, needed for risk assessment purposes. In addition, inexpensive and rapid tests that can predict tumor initiating, tumor promoting, co-carcinogenic and anticarcinogenic activities among synfuel samples are needed due to the potentially large number of feedstocks, conversion processes and process streams.

The S. typhimurium histidine reversion bioassay (the Ames assay) (3,4) utilizing strain TA98 and a rat liver enzyme preparation has been only partially successful as a short-term bioassay for detecting tumor initiators and/or complete carcinogens in synfuels as mutagens. Although positive correlations between the microbial mutagenicity and the relative tumor initiating capacity of synfuels and related materials have been observed (5,6), "false negatives" have been reported. For example, a hydrotreated Paraho shale oil has been reported to be carcinogenic (7-10) but not mutagenic

(9-12). Microbial mutagens and carcinogens have both been reported to be concentrated in the highest boiling distillation fractions from synthetic fuels (5, 12-19). However, the microbial mutagenicity of synfuel chemical class fractions has been found to reside primarily in fractions enriched in amino-substituted polycyclic aromatic compounds (20,21). Neutral polycyclic aromatic hydrocarbon-enriched synfuel fractions, which have shown relatively high carcinogenic activity, have demonstrated little or no microbial mutagenicity (17,22). Based upon these latter results, Holland et. al. (22) have suggested that with synfuels, the "apparent correlation between Ames mutagenicity and mammalian skin tumorgenicity may be due to the coincidental occurrence of two dissimilar classes of molecules."

Coal- and shale-derived liquids have been known to possess potent phototoxic properties for many years (i.e., they enhance the lethality of non-ionizing radiation) (23). In addition, a positive correlation has been reported to exist between the phototoxic and carcinogenic activities of polycyclic aromatic compounds (24-27). More recently, a number of synfuel materials have been shown to possess photomutagenic activity, i.e., the ability to enhance the mutagenicity of non-ionizing radiation (11,28-34). However, the significance of this photomutagenic activity towards potential occupational hazards and the role of photomutagens in effects seen in mammals are not yet clear. In an effort to determine the chemical nature of photomutagens in synthetic fuels and to determine the relationship between photomutagenic and carcinogenic activities, we have begun testing synfuel fractions (isolated based upon boiling point and chemical class) that have been chemically characterized and tested for skin tumor initiating capacity. We report here our progress to date and preliminary conclusions.

MATERIALS AND METHODS

Samples

Samples included coal-derived materials from the Solvent Refined Coal-I (SRC-I) and SRC-II processes. An SRC-II blend of recycle process solvent and atmospheric flash column bottoms was collected at the Harmarville, PA process development unit and was distilled by Gulf Science and Technology Co. into the following boiling point cuts (^{o}F): 300-700, 700-750, 750-800, 800-850, and 850+. An SRC-I process solvent was collected from the Wilsonville, AL pilot plant operated by Catalytic, Inc. and Southern Company Services and was distilled by Air Products, Inc. (Linwood, PA) into boiling point cuts which included an 800^{o}F+ cut, the only SRC-I cut used in this study. (Sample descriptions are summarized in ref. 17).

A crude Paraho shale oil and a hydrotreated Paraho shale oil were obtained from the Oak Ridge National Laboratory Synfuels Research Materials Repository (Repository ref. # 4601 and 4602, respectively). The crude shale oil was produced by the Paraho Development Corp. at Anvil Points, CO and hydrotreated at Sohio's Toledo (OH) refinery. The densities of these samples are reported to be 0.917 g/ml (crude) and 0.842 g/ml (hydrotreated) (44). A more complete description of the shale oil samples is available in ref. 35.

The coal oil and shale oil samples were acquired from pilot plant or process development unit test facilities and should not

necessarily be considered to be representative of synthetic fuel materials that may eventually be produced at commercial-scale facilities.

Chemical Class Separation

The hydrotreated Paraho shale oil sample was chemically fractionated prior to bioassay using the procedure of Later et al. (36). The separation procedure involved adsorption of approximately 0.1-0.2 g of sample onto 3 g of neutral alumina, packing the alumina onto 6 g of fresh alumina, and successive elution with hexane (fraction A1), benzene (A2), chloroform:ethanol (99:1)(A3) and 10% ethanol in tetrahydrofuran (A4) to give fractions enriched in aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH), nitrogen-containing polycyclic aromatic compounds (NPAC) and hydroxy-substituted polycyclic aromatic compounds (HPAC), respectively. The coal-derived samples were fractionated using a slightly modified procedure which has been routinely used at Pacific Northwest Laboratory to generate fractions for chemical and biological analysis. The alumina was activated by storing at 150°C, the water content of the alumina was maintained at approximately 1.5% and methanol replaced ethanol:tetrahydrofuran as the final eluent. Also, the sample-adsorbed (3g) and fresh alumina (11g) were solvent-packed with hexane (37,38). It should be noted that oxygen and sulfur heterocyclic compounds elute primarily with the neutral PAH-enriched fraction (A2) using these procedures (36).

Preparation of Samples for Photomutagenicity Testing

Synfuel samples were diluted and mixed in DMSO (Sigma, Grade 1) prior to bioassay. Most of the materials tested, including the A2-A4 chemical class fractions, were completely soluble in DMSO; however, when some of the coal oil distillate cuts were diluted in DMSO, a small amount of insoluble material was observed. A considerable portion of the crude and hydrotreated shale oils appeared to be DMSO-insoluble. In all cases the DMSO-insoluble components had the same appearance (waxy, aggregating particles) as the aliphatic hydrocarbon fractions (A1) diluted in DMSO. The insoluble materials present in the DMSO preparations of the SRC crude distillate cut materials and the shale oils were assumed to be the aliphatic hydrocarbon components. The aliphatic hydrocarbon fraction weight percent compositions of the SRC-II 300-700°F, 700-750°F, 750-800°F, 800-850°F and 850°F+ distillates have been reported to be 32, 15, 15, 4 and 2%, respectively (39). The crude and hydrotreated Paraho shale oils have been reported to contain 45 and 16% DMSO-extractable material, respectively (29). An aqueous solution of an aliphatic hydrocarbon-enriched (A1) fraction was prepared using the detergent Tween 80 (Sigma) and methods previously described (40-42). In brief, an aqueous preparation was made with the A1 fraction at 2 mg/ml and 20% (v/v) Tween 80 in distilled water. Bacterial suspensions were exposed to the test substances by adding 0.1 ml of the aqueous or DMSO preparation to 3.9 ml of bacterial suspension, giving final treatment concentrations of either 2.5% DMSO (v/v) or 0.5% Tween 80 (v/v). In treatments with DMSO preparations having an insoluble aliphatic hydrocarbon component, the actual oil concentration in solution would be reduced in proportion to the amount of DMSO-insoluble (aliphatic) material present.

Bioassay

Photomutation assay procedures were performed essentially as described previously (11) and were slight modifications of the method of Ames et al. (3) and Maron and Ames (4). Suspensions of Salmonella containing 1-2x10^9 cells/ml in phosphate buffer (0.1 M, pH 7.4) were untreated or treated with 1) fluorescent radiation, 2) test substance (in the dark), or 3) fluorescent radiation and test substance, concurrently. Fluorescent radiation was from General Electric and Philips (Westinghouse) 15 Watt cool white fluorescent tubes with an irradiance to the suspensions of 18 W/m^2. (The irradiance was 17 W/m^2 in tests of the unfractionated shale oils.) Following predetermined durations of exposure to test substance and/or radiation, 0.1 ml volumes of treated suspensions were removed for measurements of mutation (as reversion to histidine prototrophy) or survival. Histidine reversion was measured using the plate incorporation method (3) with top agar supplemented with 0.1 ml of nutrient broth and insufficient histidine for growth of non-revertants to macroscopic colonies. Survival was measured following dilution in nutrient broth by plating 0.1 ml volumes using the plate incorporation method (3) with top agar supplemented with 0.15 ml of 0.1 M histidine HCl (Sigma). Strain TA98 was used in all experiments.

The Salmonella/mammalian-microsome test procedure of Ames et. al. (3) and Maron and Ames (4) was used with minor modifications as described in ref. 33. Strain TA98 was used in all experiments.

Revertant and surviving colonies were counted following 2-3 days incubation at 37.5°C. The spontaneous number of revertants/plate observed in the absence of treatment was subtracted from revertant/plate values observed on mutation assay plates to give corrected revertant/plate values for each treatment. Based upon the number of corrected revertants/plate and the corresponding number of survivors for each treatment, the mutation frequency response (in revertants/10^9 survivors) was calculated by the method of Green and Muriel (43). The mutation frequency response to fluorescent light (in the absence of test substance) was subtracted from the mutation frequency responses to light and test substances to give the corrected revertants/10^9 survivors (plotted in the text figures). Text Figures 2 and 3 and Tables 1 and 2 give means and standard deviations for values obtained from multiple, indpendent experiments (where they can be plotted).

RESULTS

Effect of Hydrotreatment on the Mutagenicity of Shale Oil

Crude and hydrotreated Paraho shale oil samples were tested for mutagenicity 1) in the Salmonella/mammalian-microsome test and 2) in the photomutation assay. When the parent crude and hydrotreated oils were tested in the Salmonella/mammalian-microsome test in the absence of microsomal enzymes and in the photomutation assay in the absence of light, mutagenic responses to the oils were not detected; direct-acting mutagens were not detected in either oil. When the oils were tested using a microsomal enzyme preparation (S9), the results shown in Figure 1 were obtained. These results (Fig. 1) are in agreement with other studies (9-10,12) in showing that the crude Paraho shale oil is mutagenic and the hydrotreated shale oil is not detectably mutagenic towards Salmonella when tested in the presence

of rat liver microsomal enzymes (i.e., the Ames assay). Slope values (revertants/ug) for the responses shown in Figure 1 were calculated using data from initial linear regions of dose-response curves and are given in Table 1 (in the "Ames assay" column).

The photomutagenic responses of Salmonella suspensions to fluorescent light plus either the crude shale oil or the hydrotreated product oil are shown in Figure 2. The shale oils were tested at several concentrations and the mutation frequency responses are plotted in Figure 2 as a function of the product of shale oil concentration times duration of irradiation. The apparent dependency of the photomutagenic responses on the product of oil concentration times light exposure demonstrates a form of "reciprocity" of oil and light doses on the photomutagenic response, a phenomenon observed previously with an Eastern U.S. shale oil sample (34). The responses shown in Figure 2 were normalized to reflect the response to 100 ug/ml and slope values for linear fits of the mutation frequency responses as a function of minutes irradiated are given in Table 1. Based upon these slope values, hydrotreatment reduced photomutagenicity by approximately 78%.

Photomutagenicity of Hydrotreated Paraho Shale Oil Chemical Class Fractions

Column chromatography of the hydrotreated Paraho shale oil on neutral alumina (as described in refs. 36-38) yielded fractions termed A1, A2, A3 and A4 which were enriched in aliphatic hydrocarbons, neutral polycyclic aromatic hydrocarbons (PAH), nitrogen-containing polycyclic aromatic compounds (NPAC), and hydroxy-substituted PAC (HPAC) respectively. Fractions from several chemical class fractionations were bioassayed; the total recovery of material eluted by alumina column chromatography was > 84%. The percentage of the original material recovered in each fraction was: A1, 70-71%; A2, 10-11%; A3, 1%; and A4, 2-10%. (Values represent the range of recoveries from multiple determinations.) These results are in reasonably good agreement with values reported for a different separation procedure; i.e., 66.5% saturates, 9.8% PAH, 5.4% NPAC, 0.9% polars and 82.6% total recovery (45). The differences in recoveries of polar compounds may reflect an effect of alkyl substitutions (alkylation resulting in part from hydrotreatment) on chemical class separation schemes based upon the polarity of the sample. The A2-A4 fractions were each tested for photomutagenicity using 100 ug/ml; the mutation frequency responses are shown in Figure 3. Figure 3 shows that the A2 fraction was the most photomutagenic fraction, although all three fractions were active. The recovery of fraction A4 from the shale oil was highly variable (2-10%). The photomutagenicity of the A4 fraction was generally lower when the recovery of fraction A4 was low and the data for fraction A4 plotted in Figure 3 are from an A4 fraction in which high recovery was obtained. Slope values for mutation frequency responses as a function of minutes irradiated are given in Table 1.

Photomutagenicity of Coal Oils as a Function of Boiling Point Range

SRC-II distillation cuts having different boiling point ranges were tested for photomutagenic activity using 50 ug/ml of each oil (less the DMSO-insoluble component, see Materials and Methods). Slope values for mutation frequency responses as a function of minutes irradiated are given in Table 2. The data show a trend towards higher photomutagenic activity with increasing boiling point of the

material tested. In the absence of fluorescent light irradiation, the same SRC-II cuts were not detectably mutagenic, except the 800-850°F cut, which gave responses that were suggestive of a very low level of direct-acting (non-photosensitized) mutagenic activity. Treatment with 100 ug/ml of the 800-850° cut for two hours in the dark resulted in 34 revertants per plate above background.

Photomutagenicity of Coal Oil Chemical Class Fractions

The SRC-II 800-850° distillation cut was also separated into chemical class fractions by column chromatography on neutral alumina. Fractions from several separations were bioassayed; in these separations total recovery was ≥ 98%. The percentage of the original material recovered in each fraction was: A1, 3-4%; A2, 54-55%; A3, 23-25%; and A4, 17%; values that are in reasonable agreement with previously reported values (17,39,46). Fractions were prepared for bioassay as solutions in DMSO except fraction A1, which was insoluble in DMSO and was prepared as a DMSO "slurry" and as an aqueous solution with Tween 80. The slope values representing the mutation frequency response to fractions A1-A4 as a function of minutes irradiated are given in Table 2. The A2 fraction was clearly the most photomutagenic fraction; the A3 fraction was also photomutagenic, but the A1 fraction was inactive when tested either as a DMSO slurry or as an aqueous preparation with Tween 80. The A4 fraction was only slightly active or inactive. The SRC-II A1-A4 fractions were not detectably mutagenic when tested in the dark, except fraction A3, which induced 34 revertants per plate above background following two hours of exposure.

An SRC-I 800°+ distillate was also separated into fractions A1-A4; however, only the A2 fraction was soluble in the bioassay system. The A2 fraction was tested in a preliminary experiment (data not shown) and found to have no detectable mutagenicity in the dark; however, in the presence of light the A2 fraction was highly photomutagenic, showing a level of activity similar to that induced by the A2 fraction of the SRC-II 800-850° cut (Table 2).

DISCUSSION

Although a relatively limited number of samples have been tested, our data suggest that high boiling point components in the PAH-enriched fraction are the determinant chemical photomutagen(s) in synthetic fuels. Substantial photomutagenicity was also measured in coal oil and shale oil NPAC fractions and the HPAC shale oil fraction. The HPAC and aliphatic hydrocarbon fractions isolated from the SRC materials were relatively inactive. Strniste et. al. (31) has reported similar results in which essentially all the photomutagens (measured using cultured mammalian cells) present in a shale oil retort by-product water partitioned into a base- and neutral-enriched fraction.

Chemical analyses of PAH present in the materials tested are available in the literature (e.g., 17,18,45). Numerous PAH have been identified among the samples tested, including a variety of four- and five-ring compounds and alkyl derivatives thereof, and carcinogens such as benzo(a)anthracene, methylchrysenes, benzofluoranthenes and benzo(a)pyrene. It is not yet known which compounds present in the PAH fractions were responsible for photomutagenic activity. Benzo(a)pyrene has been reported to be photomutagenic in cultured mammalian cells (32) and UV radiation can enhance (and

inhibit) the carcinogenic response of mammalian cells to benzo(a)-pyrene (47). The occurrence of carcinogens and mutagens such as benzo(a)pyrene in samples that were not mutagenic with enzyme activation (SRC-II 800-850° A2 fraction, SRC-I 800°+ A2 fraction, hydrotreated Paraho shale oil, see Tables 1 and 2) suggests the presence of antimutagens in these samples. Haugen and Peak (48) have shown that undefined components can inhibit the microsomal enzyme activation of mutagens in coal liquids. Some heteroatomic compounds can also be expected to be present in the A2 fractions. For example, an SRC-II heavy distillate (from which the 800-850°F cut was in part derived) has been shown to contain three and four ring thiophene analogs, as well as dibenzofuran and methyldibenzofuran (30). Nitrogen heterocyclics identified in the hydrotreated Paraho shale oil include alkylated and unsubstituted carbazoles, benzocarbazole(s), and azapyrene(s) (36). The NPAC fraction of the SRC-II 800-850°F cut has been reported to contain a high concentration of benzo(a)carbazole (20.5 mg/g) and lower amounts of azapyrenes, 2-azafluoranthene, and numerous amino-substituted PAH (17).

This study was conducted in part to determine the relationship between mutagenic and carcinogenic activities in complex mixtures such as synthetic fuels. Tables 1 and 2 give data indicative of carcinogenicity, photomutagenicity, and mutagenicity in the presence of microsomal enzymes (Ames assay) for shale oil (Table 1) and coal oil (Table 2) materials. Both the crude and hydrotreated Paraho shale oil samples have been reported to be carcinogenic following chronic dermal applications to mouse skin. Hydrotreatment was reported to reduce but not eliminate carcinogenicity (7-10), and the carcinogenic potencies of the crude and hydrotreated Paraho shale oils given in Table 1 are relative to the response to benzo(a)pyrene (8). The carcinogenic potencies of the coal oil materials given in Table 2 were derived from chronic and/or initiation-promotion (IP) mouse skin painting tests. The carcinogenicity results and the mutagenicity with enzyme activation (i.e., Ames assay results using strain TA98) for the coal oils (Table 2) have been normalized to give responses relative to the response to an SRC-II heavy distillate, arbitrarily given a value of 100 (39).

A comparison of the carcinogenic and mutagenic activities given in Tables 1 and 2 should be approached with caution. The data in Tables 1 and 2 were to some degree derived by procedures that provide estimates and different analyses were apparently used to arrive at the carcinogenicity values given for the shale oils, the SRC-II distillates and the SRC-II 800-850° chemical class fractions. A cautious approach for comparing the bioassay results would be to consider only whether a test substance produced positive or negative results. The most obvious discrepancy between assays was observed with the SRC-II 800-850° A2 fraction (Table 2), which was highly carcinogenic and highly photomutagenic but was not mutagenic with enzyme activation. Similarly, an SRC-I 800°+ A2 fraction (not shown in Table 2) was observed to be highly carcinogenic in the initiation-promotion test (49), highly photomutagenic in a preliminary test (see Results), but not mutagenic with enzyme activation (19). The hydrotreated Paraho shale oil (Table 1) was also found to be positive for carcinogenicity and photomutagenicity but not mutagenic with enzyme activation (9,10,12, Table 1), although one study (50) reported a low level of enzyme-mediated mutagenicity (0.24 revertants/ug) for this sample. The SRC-II 300-700° cut (Table 2) elicited an apparently "false positive" response, being apparently

negative for carcinogenicity and photomutagenicity but positive for enzyme-mediated mutagenicity. Another report (51) also found this sample to be mutagenic with enzyme activation, although two others (18,46) did not. Of the remaining samples, the crude Paraho shale oil, the SRC-II 700-750°, 750-800°, and 800-850° cuts and the 800-850° A3 fraction were all positive for carcinogenicity, photomutagenicity, and mutagenicity with enzyme activation; the SRC-II 800-850° A1 and A4 fractions were all negative in the three bioassays or gave responses that were suggestive of a low level of activity.

In summary, of 11 samples that have been tested for carcinogenicity, photomutagenicity and enzyme-mediated mutagenicity, in 7 cases there was agreement between all three assays and in 4 cases the photomutagenicity data was in better qualitative agreement with carcinogenicity than were the mutagenicity data obtained using enzyme activation. The strong agreement between photomutagenicity and carcinogenicity among the synfuel materials could be coincidental. However, the agreement is sufficiently extensive to consider possible fundamental underlying relationships. It is possible that animals exposed to the synfuels were also exposed to significant amounts of environmental radiation (such as fluorescent room light), and the positive correlation shown in Table 2 may reflect a mediation of coal oil-induced tumorigenesis by photochemical processes. Alternatively, photosensitized effects caused by chemicals such as those present in synthetic fuels may have served as a selective pressure for the evolution of the enzymes that degrade photosensitizers and apparently have a role in PAC-induced carcinogenesis. It is also possible that photomutagenesis and carcinogenesis by PAC synfuel components proceed by the same mechanism(s). One possible common mechanism could involve the participation of reactive oxygen species such as superoxide anion, which is generated 1) by endogenous cellular chromophores when irradiated with near UV light (52), 2) in human lungs in response to chronic tobacco smoke exposure (53), and 3) possibly also in mouse skin following treatment with synfuel materials.

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Table 1. Carcinogenicity and Mutagenicity of Shale Oils

Material		Carcinogenicity[a] (chronic test)	Mutagenicity Ames[b]	Mutagenicity Photomutation[c]
Crude Paraho Shale Oil		1/998	3.7±0.1	45±20
Hydrotreated Paraho Shale Oil		1/2780	-0.0004±0.0009	10±1.7
Hydrotreated Paraho Shale Oil Chemical Classes	A2	ND[d]	ND	48±9.0
	A3	ND	ND	39±8.2
	A4	ND	ND	32±7.8

[a]From ref. 8, carcinogenic potencies relative to benzo(a)pyrene.
[b]This study, revertants/ug oil.
[c]This study, revertants/10^9 survivors/minute irradiated, 100 ug/ml tested or normalized to give responses to 100 ug/ml.
[d]ND-not determined.

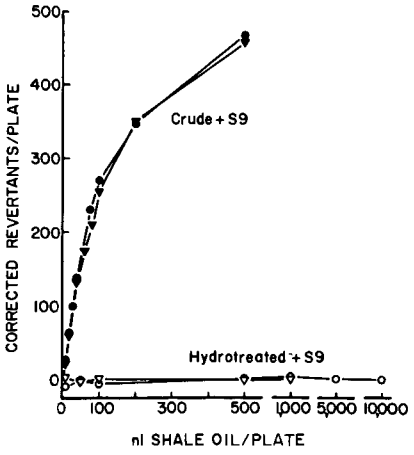

Figure 1. Mutagenicity of crude (closed symbols) and hydrotreated (open symbols) Paraho shale oils tested in two separate experiments (circles and triangles) with a microsomal enzyme preparation (S9).

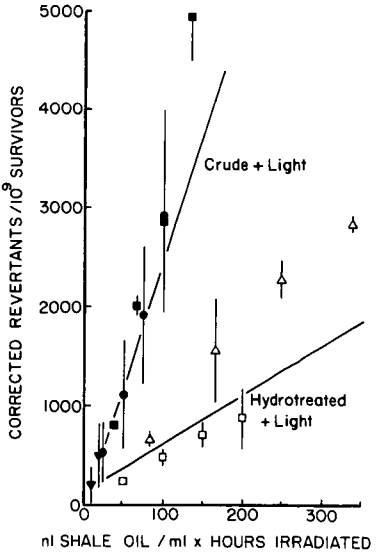

Figure 2. Photomutagenicity of crude (closed symbols) and hydrotreated (open symbols) Paraho shale oils tested using 10 (▽), 50 (○), 100 (□), or 250 (△) nl oil/ml.

Table 2. Carcinogenicity and Mutagenicity of SRC-II Distillates and Chemical Classes

Material	Relative Carcinogenicity IP[a]	Relative Carcinogenicity Chronic[a]	Relative Mutagenicity Ames[a]	Relative Mutagenicity Photomutation[b]
Distillates:				
Heavy distillate	100	100	100	ND
300-700°F	0	0	70	2.0 ± 2.3
700-750°F	18	87	95	12 ± 9.3
750-800°F	14	120	138	15 ± 3.7
800-850°F	49	157	148	27 ± 2.2
Chemical classes:				
800-850°fraction A1	10[c]		0	1.3 ± 3.8
A2	158		0	200 ± 101
A3	79		700	32 ± 9.2
A4	---[c]		0	6.6 ± 7.7

[a]From ref. 39. Values are relative to heavy distillate, given a value of 100. IP - initiation-promotion test.
[b]This study, revertants/10^9 survivors/minute irradiated, 50 ug/ml tested. ND - not determined.
[c]Not a significant response.

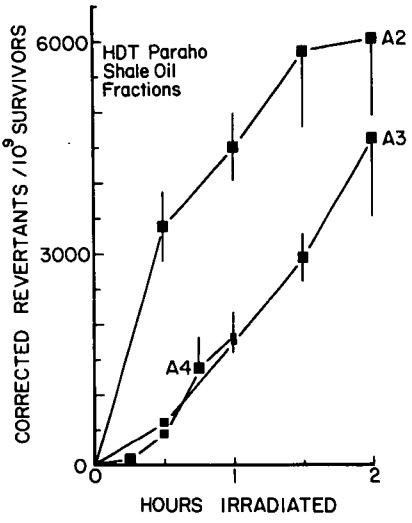

Figure 3. Photomutagenicity of hydrotreated Paraho shale oil chemical class fractions tested at 100 ug/ml.

A Ranking Tool for Potentially Carcinogenic Polynuclear Aromatic Compounds in Synfuel Products*

Tuan Vo-Dinh

Advanced Monitoring Development Group
Health and Safety Research Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831

ABSTRACT

This paper describes the use of a screening procedure to rank the content of polynuclear aromatic (PNA) species in synfuel samples. The ranking procedure is based on a cost-effective and rapid screening technique based on synchronous luminescence spectroscopy. The use of the screening procedure as a basis for screening of complex mixtures will be discussed.

INTRODUCTION

An important class of organic pollutants are PNA compounds because some of them are known to be converted by metabolic activation to ultimate carcinogens (1,2). Consequently, it is important to monitor PNA compounds in synfuel samples on a routine basis. A variety of analytical procedures have been developed to determine the concentrations of specific PNAs. High-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS) have been used to provide detailed analyses for a variety of PNAs in environmental samples (3,4). In many monitoring situations, the precise determination of various specific PNAs may be unnecessary and a prescreening phase is required to reduce the cost of environmental analysis.

This presentation describes the use of a ranking methodology that can be used to screen synfuel samples for their PNA content. The technique of synchronous luminescence (SL) is applied to fluorescence and phosphorescence measurements for establishing a ranking index (RI) for PNA species.

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EXPERIMENTAL

The Luminescence Screening Technique

The screening procedure evaluated in this study is based on the sychronous luminescence technique (5,6). In conventional luminescence spectroscopy, only one of the two spectral wavelengths, λ_{em} (emission) or λ_{ex} (excitation), is scanned while the other remains fixed. For complex samples, the resulting spectra obtained with this conventional procedure are often poorly resolved and featureless because of the spectral overlays of the emissions from individual components. However, by scanning both λ_{em} and λ_{ex} synchronously with a constant interval between the two wavelengths ($\lambda_{em} - \lambda_{ex} = \Delta\lambda$), the luminescence spectrum becomes more resolved with sharp peaks that are more readily identified.

The synchronous excitation technique can be applied both to fluorescence, i.e., synchronous fluorimetry (SF), and to phosphorescence, i.e., synchronous phosphorimetry (SP). For SF, the optimum value of $\Delta\lambda$, usually set at 3 nm, is determined by the Stokes Shift, i.e., the wavelength difference between the 0 to 0 bands in emission and absorption. For SP, the optimal value of $\Delta\lambda$ is determined by the singlet-triplet energy difference of PNA species to be monitored by room temperature phosphorimetry (RTP) (7-9).

The RTP technique is characterized by the simplicity and versatility of its methodology and consists generally of four steps: (1) substrate preparation (optional pretreatment with heavy-atom salts); (2) sample delivery; (3) drying; and (4) spectroscopic measurement. Three microliters of sample solution were then spotted on the paper circles using microsyringes with a volume of 3 microliters. Since moisture can quench the RTP emission, predrying was achieved with infrared heating lamps. Continued drying during the measurement was accomplished by blowing warm, dry air through the sample compartment. Phosphorimetric measurements were conducted with a commercial Perkin-Elmer spectrofluorimeter (Model 43A) equipped with a rotating phosphoroscope. Details on the measurement procedures have been described elsewhere (7,9).

The presence of heavy atoms in the immediate environment of the molecule can significantly enhance the population of the triplet state (external heavy-atom effect) and, therefore, the phosphorescence intensity. For PNA compounds, a large variety of heavy atom salts such as thallium and lead acetate have been found to be very efficient in enhancing the phosphorescence quantum yields. The detection limits for most PNA compounds investigated can be lowered, in some cases, by several orders of magnitude and are in the subnanogram range. It is also possible to selectively enhance the phosphorescence emission of a given compound (or group of compounds) in a complex mixture. Selective triplet emission enhancement considerably extends the specificity of the RTP technique in multicomponent analysis.

The rationale for ranking the samples for luminescence spectroscopy is based upon the fact that the majority of PNA species, especially the polyaromatic hydrocarbons, fluoresce and/or phosphoresce. Luminescence is known as two of the most sensitive techniques to detect these compounds. Provided that all the spectral interferences are accounted for, the screening procedure can be based on the principle that the higher the total intensity of the SL bands, the more concentrated the samples are in PNA content.

APPLICATIONS

Screening Profiles of Coal Liquid Samples

Figure 1 shows the synchronous fluorescence spectra of five synfuel products collected at different locations of a synfuel production facility. In order to test the capability of the rapid screening procedures, no prior attempt had been made to analyze these samples and obtain compound-specific information about the individual components. All of the five samples were diluted in ethanol by serial dilution (10^3, 10^4, 10^5, and 10^6 dilution factor). A wavelength interval of $\Delta\lambda$ = 3 nm was used in the synchronous fluoreacence measurements. With the use of the $\Delta\lambda$ value, the resulting synchronous fluorescence peaks correspond approximately to the 0,0 band emissions of most PNA compounds. The synchronous profile, therefore, is not just a spectral fingerprint, but contains useful information about the nature and PNA composition of the samples.

The main relationship between the size of the benenoid structure of a polyaromatic hydrocarbon and its fluorescence emission is the dependence of its 0,0 band upon the number of benzene rings (5). The wavelength position of the 0,0 band of a high-number, linear fused-ring size cyclic compound generally occurs at longer wavelengths than that of a lower number ring-size compound.

Synchronous fluorescence measurements were conducted with the five products at various concentrations. The results indicated that the SF profile remained unchanged when the samples were diluted to 104 fold or less. This indicated that spectral interferences did not occur at these concentration levels for the products investigated. Without any identification and quantification procedure, a rapid examination of the synchronous fluorescence profiles of the five synfuel products A, B, C, D, and E at 10^5 fold dilution levels can readily provide the following conclusions (Figure 1). A rapid comparison of the SL profiles first indicates that product C should contain the least amount of PNA compound. The compound that contributes to the peak at 285-290 nm in sample C is a monocyclic aromatic species and it is also present in similar amounts in other samples (A, B, D, and E). The intensity of the peak at about 305 nm is approximately 10-fold less intense in sample C than in the other samples. Besides a weak shoulder at about 325 nm, no other band was detected in sample C at wavelengths longer than 320 nm.

In order of increasing PNA content, product D is the next sample to consider. The peak at 305 nm is about 10-fold more intense than that of sample C. A rapid examination of the SL profile of sample A also shows that this product is similar to product B. Product B, however, contains slightly more PNA compounds that have 0,0 bands at 346 nm, 382 nm, 402 nm, and 442 nm. Although the general structure of the spectra for products A and D are similar, these spectral differences are still noticeable. Finally, the synchronous profiles show that the PNA content of products B and E are similar. These two samples contain more PNA compound with 3-5 rings than samples A, C, and D as indicated in Figures 1b and 1e.

Another example of the screening procedure by RTP is the characterization of another series of coal liquids produced by a synfuel production process. The results of this screening procedure are shown in Figure 2. All the samples were diluted in ethanol by serial dilution (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) and spotted on filter paper (Schleicher and Schuell, No. 2043A) treated with a mixture of thallium acetate and lead acetate. The excitation used to obtain the RTP spectra was 315 nm. This wavelength was used to excite most of the PNA compounds having 3 to 5 fused rings. The samples shown in this figure were 10^{-6} fold diluted. Without any identification and quantification procedure, it is possible to rank these

samples as follows: B > A > D > C. Note that sample C exhibited stronger intensity at approximately 600 to 650 nm where pyrene and other 4- to 5- ring PNA compounds mainly emit.

The above examples show that it is possible to obtain a preliminary ranking of coal products after a rapid synchronous scanning procedure. All the samples were screened without any prior prefractionation or precleaning process. Each SL and RTP measurement was conducted in less than five minutes, after the appropriate concentration range had been selected. Recently the SL technique has been developed for measuring important biomarkers including PNA metabolites and PNA-DNA adducts produced by human exposure to PNA pollutants (10,11).

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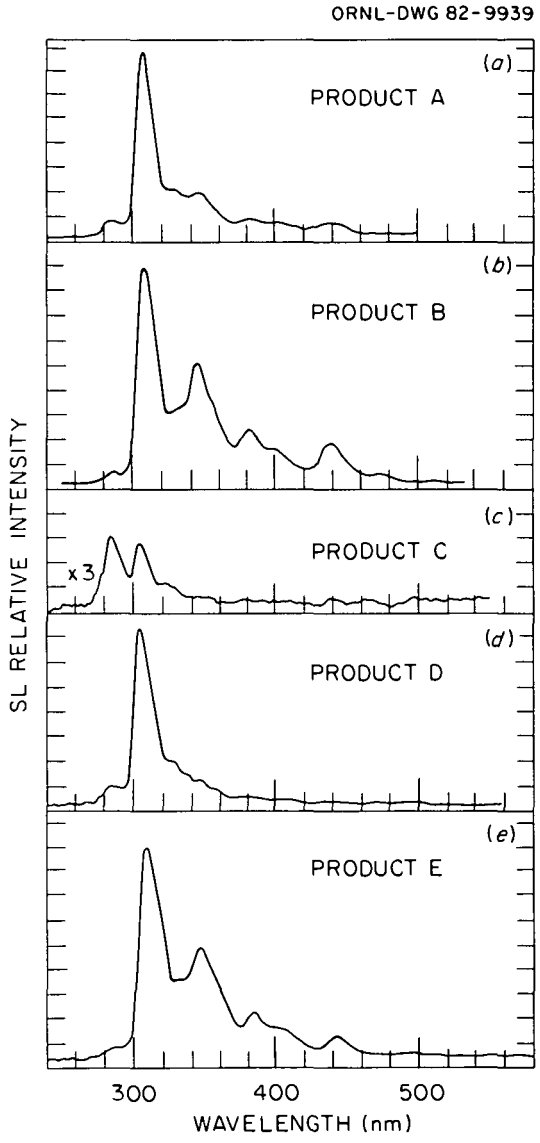

Figure 1: ***Ranking Procedure of several Coal Liquid Products by Synchronous Fluorescence.***

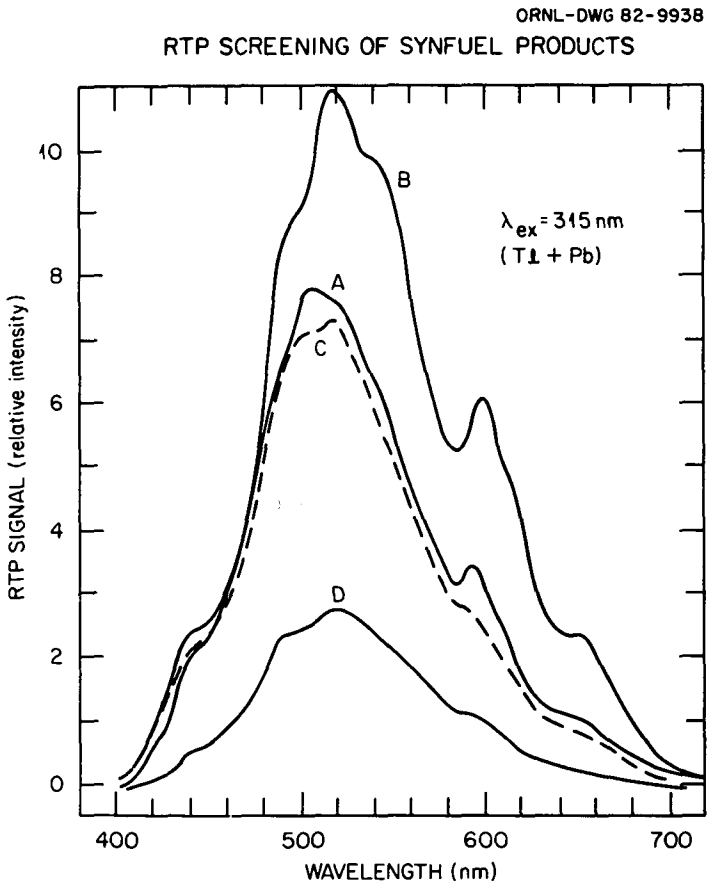

Figure 2: Ranking Procedure of Synfuels Products by Room Temperature Phosphorimetry.

Comparative Toxicity of Crude and Refined Coal Liquids and Analogous Petroleum Products: II. Chemical Characterization*

W. H. Griest[a], C.-h. Ho[a], M. R. Guerin[a], H. P. Witschi[b], and L. H. Smith[b]

Analytical Chemistry Division[a] and Biology Division[b],
Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831

Introduction

In Part I of this paper, the dermal tumorigenicity of crude and hydrogenated H-Coal Blends, a home heating oil and a reformed naphtha refined from the hydrotreated H-Coal Blend, and two analogous petroleum products is reported. This paper compares the tumorigenicity assay results with the chemistry of the samples.

Experimental

Samples: The crude and upgraded coal liquids consisted of a water-washed 60/40 (wt./wt.) blend of Light and Heavy Oils from the H-Coal Pilot Plant at Catlettsburg, KY) (H-Coal Blend-AWW, sample identification no. 931), the blend hydrotreated under "low severity" conditions resulting in 900 SCF/Bbl of hydrogen incorporation (H-Coal Blend-HDT/L, no. 934), the blend hydrotreated under "medium severity" conditions for 1400 SCF/Bbl of hydrogen consumption (H-Coal Blend-HDT/M, no. 933), and the blend hydrotreated under "high severity" conditions for 3000 SCF/Bbl hydrogen consumption (H-Coal Blend-HDT/H, no. 935). In addition, two products were prepared as petroleum product substitutes. No. 935 H-Coal Blend-HDT/H was devolatilized to meet most of the ASTM specifications for no. 2 fuel oil and was designated as H-Coal Home Heating Oil (no. 978). A 96 octane "gasoline" product (H-Coal Reformed Naphtha) was prepared by a high severity hydrogenation of the no. 931 H-Coal Blend, followed by hydrocracking and catalytic reforming. Blending, catalytic hydrogenation, and hydrocracking were conducted by the Chevron Research Corporation (Richmond, CA), and catalytic reforming was performed by Universal Oil Products, Inc. (Des Plaines, IL). Two petroleum products, API no. 2 Fuel Oil (no. 975) and API Light Catalytically Cracked Naphtha (no. 976) were supplied by the American Petroleum Institute (Washington, DC). Sample origin and processing are described in detail elsewhere (1,2).

Mouse Skin Dermal Tumorigenicity: (See Part I.)

Bacterial Mutagenicity: Bacterial mutagenicity was determined using the plate incorporation assay of Ames with strain TA-98 and Aroclor-induced S-9 metabolic activation.

Organic Chemical Characterization: Benzo(a)pyrene was measured using a sequential high performance liquid chromatography/high performance liquid chromatography (HPLC/HPLC) procedure and quinoline was estimated by direct injection of a diluted sample into a gas chromatograph (GC) equipped with a packed column and a nitrogen-compound-selective thermionic detector. The 5-ring polycyclic aromatic hydrocarbons (PAH) were estimated by capillary column GC following preparation of a PAH-enriched fraction using semi-preparative HPLC. Major organic compounds in the fuels were identified by capillary column GC-mass spectroscopy. The chemical class distribution was determined gravimetrically after acid/alkaline partitioning of the sample and gel chromatography of the neutral fraction. More detailed descriptions of these procedures are given in reference (1).

*Research sponsored by the Office of Fossil Energy, U. S. Department of Energy under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

Results and Discussion

The changes in bulk composition and properties of the H-Coal Blend as the severity of hydrogenation is increased are consistent with those found in other studies, and are not reported in detail here (see reference 1). Briefly, the heteroatomic content decreases while the hydrogen concentration increases, with S being reduced most readily and O least easily. The density and viscosity decrease, the boiling range is lowered, and both the flash point and pour point decrease with increasing severity of hydrotreatment.

The more toxicologically important compositional changes are compared in Table 1 with summaries of the mutagenicity and dermal tumorigenicity assays. It is evident that tumorigenicity and mutagenicity are dramatically reduced by hydrogenation. Mutagenicity is undetectable in the hydrogenated blends when the sample is assayed as a slurry in dimethylsulfoxide. Tumorigenicity is reduced by low severity hydrotreatment, but no further reduction is evident for the high severity hydrotreated sample. The concentrations of toxic components or chemical classes in the samples also decrease with increasing severity of hydrotreatment, but not in direct proportion to the reductions in toxicity. BaP, a classic PAH dermal tumorigen drops to less than 0.4% of its original level upon low severity hydrotreating of the blend. A more gradual decrease in concentration is noted for the polycyclic aromatics chemical class fraction, which includes the PAH dermal tumorigens. The PAH are associated with the tumorigenicity (3-5) of crude coal liquids.

Quinoline, one of the major N-heterocyclics, decreases in concentration with increasing hydrotreatment severity. The ether-soluble base chemical class fraction also decreases in concentration. This fraction includes the polycyclic aromatic primary amines, which are the determinant mutagens in crude coal liquids (6,7).

In contrast, phenol appears to resist reduction until high severity hydrogenation conditions are employed. This behavior parallels the relative difficulty in reducing the total O content as gauged by ASTM Ultimate Analyses of the crude and hydrotreated blends (1). This trend is not reflected in the ether-soluble acid fraction, which contains most of the oxygenates such as phenols and carboxylic acids.

The PAH content and dermal tumorigenicity of the refined products derived from coal liquids and petroleum are compared in Table 2. It is evident that the further refining (hydrocracking and catalytic reforming) to produce the H-Coal Reformed Naphtha has eliminated the tumorigenicity of the original crude H-Coal Blend. The API Light Catalytically Cracked Naphtha is only slightly tumorigenic. The home heating oils are somewhat more tumorigenic than the naphthas, with the H-Coal Home Heating Oil being more tumorigenic than the API No. 2 Fuel Oil. It also is apparent that the least tumorigenic product (the H-Coal Reformed Naphtha) has the highest BaP and 5-ring PAH concentrations. The fuel oils have lower BaP and PAH content than does the H-Coal Reformed Naphtha, but much higher tumorigenic activities.

One hypothesis for this apparent disparity between PAH content and tumorigenicity is that there are differences in tumor promoting activity among the samples. This hypothesis is being tested. An alternate hypothesis is that the expression of tumorigenicity by the PAH is mediated by differences in the sample matrix composition. The capillary column GC separations shown in Figure 1 illustrate that major compositional differences do exist between the H-Coal Home Heating Oil and the API No. 2 Fuel Oil. Mass spectral analysis of the samples confirmed these differences. The API No. 2 Fuel Oil is composed mainly of C_7-C_{24} n-alkanes and alkylated 1-3 ring aromatic hydrocarbons (in order of decreasing concentration: C_0-C_5-naphthalenes, C_0-C_4-benzenes, C_1-C_4-indanes, and C_0-C_1-phenanthrenes). In contrast, the H-Coal Home Heating Oil is comprised mainly of cycloparaffins (in order of decreasing concentration): decahydronaphthalene, C_1-C_4-decahydronaphthalenes, C_0-C_4-cyclohexanes, and C_0-C_4-tetralins. Aromatic and partially saturated aromatic hydrocarbons

and alkanes also are present, but at much lower levels (in order of decreasing concentration): C_1-C_4-indanes, C_7-C_{25} n-alkanes, and C_0-C_4-benzenes.

The chromatograms of the naphthas (Figure 2) show that they are quite different as a group from the home heating oils. The two naphthas share many components, such as C_4-C_7-alkanes and C_0-C_3-benzenes. However, the H-Coal Reformed Naphtha is more aromatic than the API Light Catalytically Cracked Naphtha, having 7-fold more benzene, 4.5-times more toluene, and 2-fold greater levels of C_2-benzenes. In contrast, the API Light Catalytically Cracked Naphtha is more olefinic, being more enriched in partially unsaturated C_5-C_8 hydrocarbons. The fluorescent indicator assay results reported by the Universal Oil Products, Inc., and the American Petroleum Institute reflect these compositional differences: H-Coal Reformed Naphtha (58.4% [vol./vol.] aromatic, 0.9% olefin, 40.7% saturate) and API Light Catalytically Cracked Naphtha (20.3% aromatic, 29.6% olefin, 50.0% saturate). It is possible that these compositional differences among the samples affects the absorption, metabolism, and uptake of PAH tumorigens, and modifies or potentiates their tumorigenic activities.

Conclusions

The decrease in tumorigenicity of the crude H-Coal Blend by catalytic hydrogenation is associated with the reduction of tumorigens. Comparison of the composition and residual tumorigenicity in refined products derived from the H-Coal Blend and from petroleum suggests that the tumorigenicity associated with PAH is modified by the presence of tumor promoting agents, by matrix composition differences, or both.

Acknowledgement

The American Petroleum Institute (API) is gratefully acknowledged for supplying the two petroleum-derived samples.

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Table 1. Influence of Hydrotreatment on Chemical Parameters, Mutagenicity, and Tumorigenicity of Coal Liquids (1)

Sample Number	Description	Chemical Class Fraction, wt. %					Quinoline, µg/g	Phenol, mg/g	Benzo(a)pyrene, µg/g	Mutagenicity[b], rev/µg	Animals w/Skin Tumors[c], % Dose (wt./vol. %)	
		ESB	ESA	Sat.	Aro.	Polyaro.					100	50
931	H-Coal Blend - AWW	3.3	1.5	30.8	56.7	5.8	164	0.15	50	4.9	82	90
934	H-Coal Blend - HDT/L	2.4	0.8	38.3	46.5	1.3	6	0.13	<0.2	<0.1	14	12
933	H-Coal Blend - HDT/M	0.6	0.6	35.9	46.7	0.8	<1	0.13	<0.2	<0.1	-	-
935	H-Coal Blend - HDT/H	0.7	0.5	58.9	8.3	0.5	<1	<0.1	<0.1	<0.1	20	34

[a]ESB = ether-soluble bases, ESA = ether-soluble acids, Sat. = saturates, Aro. = mono/di-aromatics, Polyaro. = polycyclic aromatics.

[b]TA-98 + S-9 activation.

[c]Data for 104 weeks of three-times weekly treatment of 25 male plus 25 female C3H mice per dose group/substance. Male and female mice data combined.

Table 2. Comparison of PAH Content and Tumorigenicity of Final Products Refined from Coal Liquids and Petroleum

Sample Number	Description	Benzo(a)pyrene µg/g	Sum of 5-Ring PAH[a], µg/g	Animals w/Skin Tumors[b], % Dose (wt./vol. %) 100	50	Median Time-To-Tumor[c], Wks
936	H-Coal Ref. Nap.	1.4	35	0	0	155-ND
976	API Lt. Cat. Cr. Nap.	<0.002	<0.5	6	8	125-143
978	H-Coal Home Ht. Oil	0.08	3	28	24	97-112
975	API No. 2 F. O.	0.04	0.09	12	14	120-144

[a]Benzo(b/j)fluoranthenes + benzo(e)pyrene + benzo(a)pyrene + indeno(1,2,3-cd)pyrene + benzo(ghi)perylene.

[b]Data for 25 months of three-times weekly application to 25 male + 25 female C3H mice per dose group/substance. Acetone solvent for 50% dose.

[c]Range including all dose groups and both sexes. ND = none detected (for zero tumor incidence).

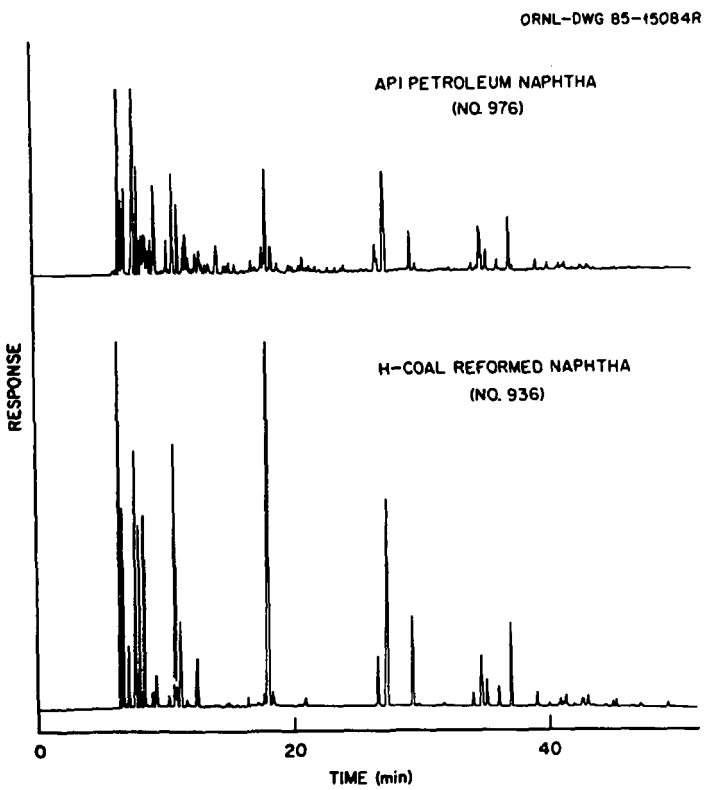

Figure 1. Capillary Column GC Comparison of Major Organic Compounds in Coal- and Petroleum-Derived Naphthas.

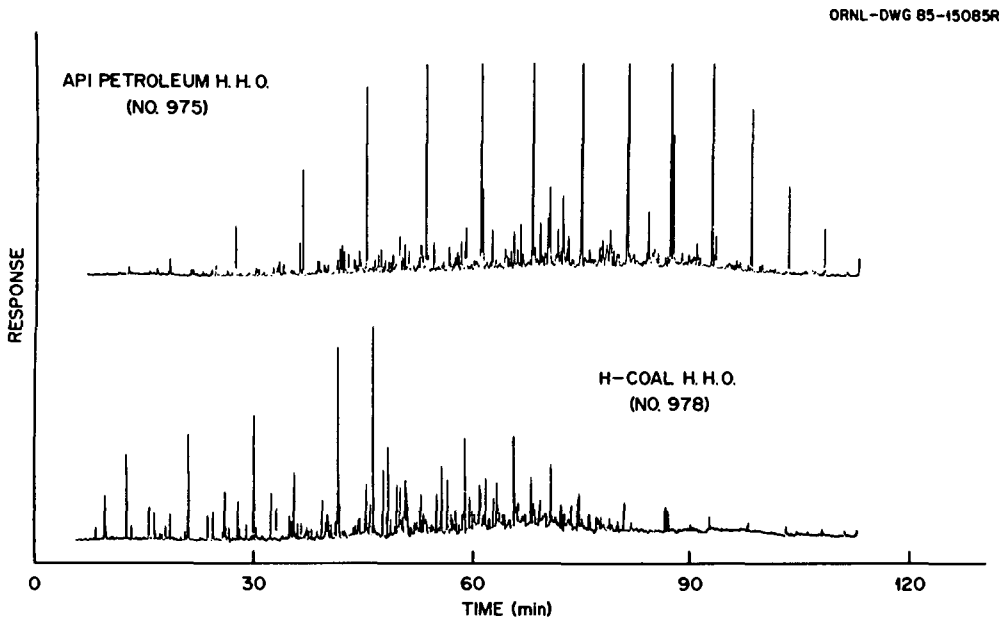

Figure 2. Capillary Column GC Comparison of Major Organic Compounds in Coal- and Petroleum-Derived Home Heating Oils.

PROCESS RELATED EFFECTS ON THE CHEMICAL AND TOXICOLOGIC CHARACTERISTICS OF COAL DERIVED FUELS

C. W. Wright,[1] E. K. Chess,[2] R. M. Bean,[2]
D. D. Mahlum,[2] D. L. Stewart,[2] and B. W. Wilson[2]
[1] Chemical Technology Department
[2] Biology and Chemistry Department

Pacific Northwest Laboratory,
P.O. Box 999, Richland, WA 99352

ABSTRACT

As a component of an ongoing program to assess the potential health effects of coal conversion materials, we have recently completed chemical and toxicologic studies of a sample set collected on selected days of a 25-day demonstration run of a catalytic two stage direct coal liquefaction (CTSL) process. There was an increase in heteroatomic, nitrogen containing polycyclic aromatic compounds (NPAC) and hydroxy-substituted PAC, compounds as the operation time of the pilot plant increased. The proportion of material which boiled above 975°F also increased in the solids-free portion of the recycle slurry oil as pilot plant operation time increased. As anticipated from the increase in NPAC concentration during the run, the microbial mutagenic activity of selected process materials also increased as a function of run time. Likewise, the tumorigenicity of the materials produced later in the demonstration run was higher than that of those produced initially. These results support the view that catalyst deactivation during the course of the run gives rise not only to lower coal conversion, but also to increased toxicologic activity.

INTRODUCTION

Chemical and toxicologic characteristics of direct coal liquefaction materials are highly dependent on the specific process by which the coal liquids were produced. Of particular importance are those process variables which can

affect the boiling point (bp) range and degree of hydrogenation of product materials (Wilson et al., 1986). Thus, process modifications such as recycle of heavy ends to extinction (Anderson and Freel, 1983), adjustment of product distillation upper temperature cut points to ca. < 650 °F (Pelroy et al., 1985), incorporation of a catalytic hydrogenation step (Wilson et al, 1986), and, to a lesser extent, changes in feed coal type can reduce the toxicologic potential of coal liquefaction materials as determined by microbial mutagenicity testing and mouse skin tumorigenesis assays.

We have recently concluded studies that assessed the effects of process run time, and hence catalyst age, on the chemical composition and toxicologic activity of materials from an advanced coal liquefaction process. In the present paper, these results are reported and compared to data from earlier studies which dealt with other process variables such as those listed above. Studies of catalyst effects on toxicology will be important in the new petroleum resid/coal co-processing schemes, which will depend heavily on catalyst performance.

EXPERIMENTAL

During July of 1984, Hydrocarbon Research, Inc. (HRI) performed a 25-day demonstration run of their catalytic two-stage liquefaction coal conversion (CTSL) process at Lawrenceville, NJ. An objective of this CTSL process was to take advantage of catalytic reactions in both reactors to convert coal to a liquid; details of the CTSL process are given by Comolli et al. (1984) and Wright and Later (1985). Samples of the pressure filter liquid (PFL; the solids-free portion of the recycle slurry oil, an internal process stream material) were taken each day of the demonstration run. A product distillate blend (PDB), more representative of the actual net product from the CTSL process, was also supplied by HRI.

Chemical analyses were performed on fractions isolated from the PFL and PDB materials by adsorption column chromatography (Later et al. 1981). Chemical class fractions of aliphatic hydrocarbons (AH), polycyclic aromatic hydro-

carbons (PAH), nitrogen-containing polycyclic aromatic compounds (NPAC), and hydroxy-substituted PAH (hydroxy-PAH) were produced using neutral alumina as an adsorbent. Due to the high concentration of hydrogenated and partially hydrogenated components present in these samples, the hydroaromatic compounds were isolated using picric-acid-coated alumina as an adsorbent (Wozniak and Hites, 1983). Selected chemical fractions isolated from the PFL and PDB materials were then analyzed by high-resolution gas chromatography (HRGC), gas chromatography/mass spectrometry (GC/MS), and low-voltage probe-inlet mass spectrometry (LVMS).

The mutagenic response of all crude samples and chemical class fractions were measured using the histidine reversion microbial mutagenicity test with Salmonella typhimurium, TA98 (Ames et al., 1975). Selected crudes were tested for tumorigenic potential using the initiation/promotion (I/P) assay for tumorigenicity in mouse skin (Mahlum, 1983). Details regarding the methods of chemical analyses and measurement of toxicologic activity in the CTSL materials are given by Wright and Later (1985).

RESULTS AND DISCUSSION

Chemical Analysis

The distillation weight percent distribution of the CTSL PFL materials from days 5, 10, 15, 19, and 24 are given in Table 1. The composition boiling above 975°F increased significantly over the duration of the demonstration run. There was a concurrent decrease in the composition which distilled less than

TABLE 1. Distillation Data for Selected PFL Sample Materials

Distillation Temperature	Weight Percent Composition				
	Day 5	Day 10	Day 15	Day 19	Day 24
IBP - 650°F	28.1	21.0	18.9	14.9	13.1
650 - 850°F	33.4	32.7	30.9	31.8	30.9
850 - 975°F	11.4	12.7	13.8	8.7	6.8
975°F+	27.1	33.6	36.4	44.6	49.2

650°F. The increase in higher boiling constituents of the PFL may be an effect of catalyst deactivation and recycle oil boiling point adjustment.

Elemental analysis data indicated there were changes in the PFL composition during the course of the 25-day demonstration run. These changes included a general decrease in the carbon content of the PFL material and an increase in the heteroatom content with catalyst age and duration of the run. The nitrogen content increased gradually from 0.61 weight percent on day 5 to 0.99 weight percent on day 24; the sulfur content increased similarly from 0.046 to 0.186 weight percent for the same days of pilot plant operation.

The chemical class composition (as determined by alumina column chromatography) of the PFL materials from days 1, 5, 10, 15, 19, and 24 are shown in Figure 1. As the demonstration run progressed, the PFL AH composition decreased by more than a factor of two, the PAH composition of the PFL materials was fairly constant, and both the NPAC and hydroxy-PAH fractions showed significant increases. The PDB had a significantly higher AH

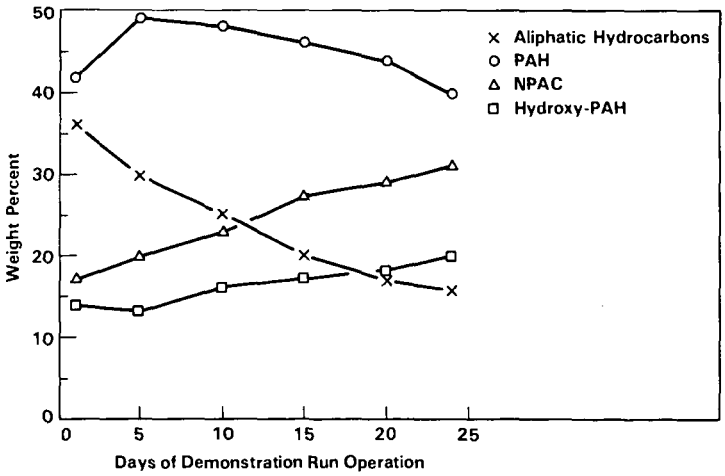

FIGURE 1. Chemical Class Composition of CTSL Pressure Filter Liquid Samples as Determined by Alumina Column Chromatography

composition and significantly lower PAH, NPAC, and hydroxy-PAH composition than did the PFL material. The PDB contained 43% AH, 23% PAH, 5.2% NPAC, and 6.7% hydroxy-PAH by weight as determined by alumina column chromatography.

The hydroaromatic composition of the CTSL PFL materials from days 1, 5, 10, 15, 19, and 24 is shown in Figure 2. The AH of fraction PA1 and the hydroaromatic compounds of fraction PA2 generally decreased as the operation time of the demonstration run increased. The dihydro- and less-than-three-ringed PAH composition of fraction PA3 was constant after about day 5 of the demonstration run. In addition, the greater-than-three ringed PAH and some slightly polar compounds of fraction PA4 generally increased with increasing operation time. Indan, tetralin, and hydrogenated acenaphthylenes, fluorenes, phenanthrenes, fluoranthenes, and pyrenes were detected as major components in the hydroaromatic fraction of the PDB when analyzed by HRGC and GC/MS. Alkylated species of each of the above were also detected in the PA2 fraction.

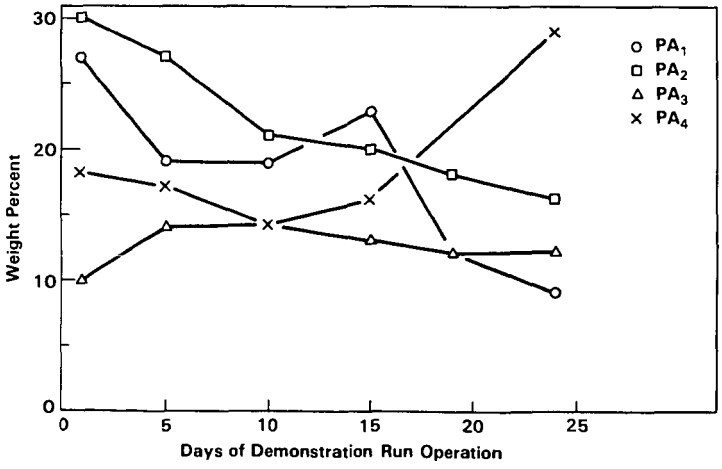

FIGURE 2. Hydroaromatic Composition of CTSL Pressure Filter Liquid Samples as Determined by Picric-Acid-Doped Column Chromatography

The PAH and NPAC fractions were analyzed in detail since these chemical class fractions have been previously shown to be the most tumorigenic and mutagenic fractions in coal-derived materials, respectively. The quantitative HRGC results for over 50 components in the PAH fractions and 40 components in the NPAC fractions of the PFL materials from day 1, 5, 10, 15, 19, and 24 are given by Wright and Later (1985). The results of the PAH fraction analyses can be summarized by the data given in Figure 3. The quantitative values for some low molecular weight PAH in the isolated PAH fractions, i.e. the methylnaphthalenes and two isomers of dimethylnaphthalene, were summed and are plotted for each of the PFL materials analyzed. The concentration of these low molecular weight components decreased over the duration of the demonstration run. The quantitative values for some of the high molecular weight PAH in the isolated PAH fractions, i.e. two methylchrysene isomers, the benzofluoranthenes, the benzopyrenes, indeno(1,2,3-cd)pyrene, and benzo(ghi)perylene, were also summed and are plotted in Figure 3 for each of the PFL materials analyzed. The

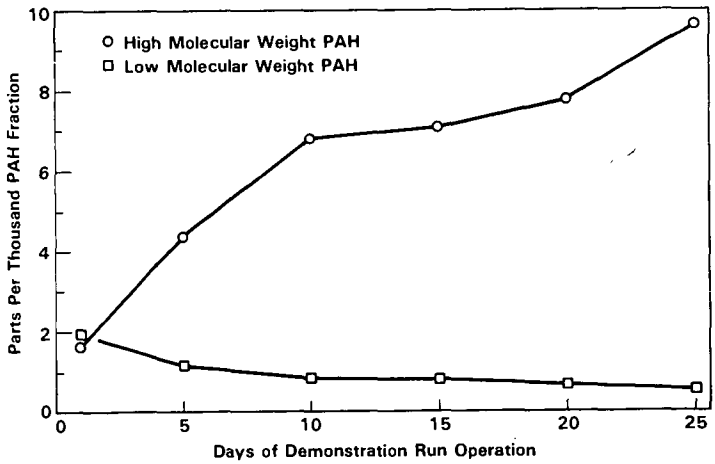

FIGURE 3. Concentrations (parts per thousand) of Selected High Molecular Weight Components and Low Molecular Weight Components in PAH Fractions of Selected CTSL PFL Samples

concentration of these high molecular weight components increased significantly with increasing pilot plant operation time. Operating temperatures were equilibrated during the first 3 days of the demonstration run; the composition of the PAH fractions continued to change after operating temperatures were equilibrated at day 3. These changes may be an effect of catalyst degradation or an effect of the resid recycle. Similar increases in higher molecular weight composition were noted for the AH, NPAC, and hydroxy-PAH chemical class fractions when analyzed by LVMS.

The individual components in the chemical class fractions of the PDB were similar to those found in the PFL materials, only for a lower molecular weight range. The quantitative analyses of the PDB and the PFL composite revealed that the concentration of the methylnaphthalenes and two dimethylnaphthalene isomers was more than an order of magnitude higher in the PDB as compared to the PFL, for example.

To compare the degree of alkylation of the CTSL PDB to products from other coal liquefaction processes, the quantitative values for the following compounds were summed and were then divided by the quantitative values of their respective parent compounds: the methylphenanthrene isomers, the methylcarbazole isomers, and 1-methyl pyrene. The resulting ratios were then summed. The summed ratios of these quantitative values are given in Table 2 for the CTSL PDB as well as solvent refined coal (SRC)-I, SRC-II, H-Coal, EDS, integrated two-stage liquefaction (ITSL) (Lummus), ITSL (Wilsonville), and nonintegrated two-stage liquefaction (NTSL) products. The single-stage, noncatalytic SRC materials showed the lowest degree of alkylation compared to the single-stage, catalytic H-Coal, hybrid EDS, or any of the two-stage coal liquefaction products. The highest degrees of alkylation were present in the two-stage coal liquefaction materials. Both ITSL, the NTSL, and the CTSL materials showed similar degrees of alkylation to each other, as determined by these means.

Those processes which incorporated catalysts had higher degrees of hydrogenation than did the noncatalytic processes. Quantitative values for the 1,2-dihydrophenalene and a dihydrofluoranthene were divided by the quantitative values of fluorene and fluoranthene, respectively, to compare the degree of hydrogenation of coal liquefaction process materials. These two ratios were

TABLE 2. Relative Degrees of Alkylation and Hydrogenation of Coal Liquefaction Materials as Determined by HRGC Analysis

Coal Liquefaction Process	Relative Degree of Alkylation[a]	Relative Degree of Hydrogenation[a]
SRC-I	1.3	0.2
SRC-II	1.7	0.6
H-Coal	3.2	4.2
EDS	3.4	4.4
ITSL Lummus	4.6	6.3
ITSL Wilsonville	4.4	6.0
NTSL Wilsonville	4.9	11
CTSL PDB	4.1	10

(a) See text for explanation.

then summed and are also given for each of the coal liquefaction materials in Table 2. The CTSL PDB and NTSL product showed the highest degree of hydrogenation, as determined by this method.

Biological Testing

The microbial mutagenicity results indicated a general trend of increasing mutagenicity with increased pilot plant operation time. The results of testing crude PFL materials from each day of the demonstration run were as follows: the responses, in rev/μg, ranged from approximately 1 to 2 for the first 5 days, 2 to 3 rev/μg for the next 5 days, 4 to 6 rev/μg for days 11 through 15, 6 to 10 rev/μg for days 16 through 20, and 9 to 12 rev/μg for the last 5 days of the demonstration run. The trend of increasing mutagenic response with time of operation correlates with the chemistry analysis data, which showed that the PFL materials had an increasing heteroatomic (particularly NPAC) composition as the length of pilot plant operation time increased. The microbial mutagenic response of coal liquefaction materials has historically been related to the NPAC chemical class fractions of coal liquefaction materials analyzed using these methods.

The CTSL PDB had a microbial mutagenicity dose response of 1.7 rev/μg with S. typhimurium, TA98. This response was higher than materials representative of Wilsonville (bp >450°F) and Lummus (bp >400°F) ITSL final products: approximately 2 rev/μg for the CTSL versus 0 rev/μg for the ITSL materials. The PDB did, however, have a lower mutagenic response than that of a NTSL (bp >450°F) final product (6 rev/μg). The majority of microbial mutagenicity of all the CTSL materials tested was associated with the NPAC fractions when the chemical class fractions were tested with S. typhimurium, TA98. These results were consistent with other coal liquefaction materials studied to date.

The results are given in Table 3 for the I/P mouse skin tumorigenicity assay of days 5, 15, and 24 PFL materials from the 25-day demonstration run of the HRI CTSL process. The mean number of tumors per mouse data (normalized to a population of 30 mice) indicated a general trend of increasing tumorigenicity with increasing length of pilot plant operation and catalyst deactivation. These I/P results were in general agreement with the chemistry results that showed that the PFL materials had decreased AH content and increased molecular weight distribution with increasing length of pilot plant operation. The percent tumor incidences were similar for all the PFL materials tested

TABLE 3. I/P Results (mean number of tumors per mouse) for Selected Coal Liquefaction Materials

Sample	Nominal bp Range (°F)	Tumors/Mouse[a]
CTSL PFL, Day 5	500 - 975+	1.37
CTSL PFL, Day 15	500 - 975+	1.62
CTSL PFL, Day 24	500 - 975+	2.27
CTSL PDB	<850	0.57
ITSL Second-Stage Product (Wilsonville)	450 - 850+	1.3
NTSL Second-Stage Product (Wilsonville)	450 - 850+	1.1
ITSL TLP (Lummus)	~400 - 850+	2.6

(a) Normalized to 30 mice per test material.

(approximately 70% after 191 days); however, day 5 results were slightly lower (~62%) and day 15 results were slightly higher (~75%) than the rest.

The skin-tumor initiating activity of the PDB (also given in Table 3 in terms of mean number of tumors per mouse) was significantly less than that of any of the PFL materials tested when judged by either tumor yield or tumor incidence; this is predictable from the significantly increased bp range of the PFL (>975°F) versus that of the PDB (<850°F) materials. Increasing tumorigenicity has been noted with increasing bp of coal liquefaction materials by Wright et al. (1985).

Also included in Table 3 are the mean number of tumors per mouse for the ITSL and NTSL materials, with nominal bp information for all samples. The CTSL PDB appeared to have less tumor initiating activity than did the NTSL or ITSL products, probably an effect of the lower bp of the former versus the latter.

ACKNOWLEDGMENT

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